VOLUME III, NO. 1 WHOLE NO. 13

JANUARY, 1927

THE AMERICAN JOURNAL OF PATHOLOGY

Official Publication of
The American Association of Pathologists and Bacteriologists

Editorial and Publication Office
818 HARRISON AVENUE, BOSTON, MASSACHUSETTS
Issued Bimonthly — Annual Subscription in U. S. A. $5.00

Entered as second class matter, March 20, 1914, at the Post Office at Boston, Massachusetts, under the Act of March 3, 1879

CONTENTS

PRINTED AT THE HARVARD UNIVERSITY PRESS, CAMBRIDGE, MASS., U. S. A.

THE AMERICAN JOURNAL OF PATHOLOGY

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STUDIES ON THE ISLANDS OF LANGERHANS IN HUMAN PANCREAS *

I. THE RELATION OF THE ISLANDS TO THE SURROUNDING STRUCTURES

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INTRODUCTION AND HISTORICAL REVIEW

The question as to the morphologic relation of the islands of Langerhans to the surrounding pancreatic structures in the human and animal pancreas is still under discussion in the literature. One group of investigators (Helly,[7] Weichselbaum and Kyrle,[23] Opie, [17] Flint [4] and others) claims that the islands generally have a limiting fibrous capsule and are, therefore, anatomically independent from the rest of the pancreatic tissue. The other group (Gellè,[6] v. Hansemann,[8] Vincent and Thompson,[21] Koch,[13] Herxheimer,[10] Fahr [5] and others) however, maintains that the islands are throughout directly connected with the surrounding acini of the pancreas. Whereas Weichselbaum and Kyrle [23] and Helly [7] concede that there are a few islands which are continuous with the ducts, Laguesse [14] and Bensley [1] describe a constant continuity with the ducts as well as with the acini.

In the most recent literature Nakamura [16] claims that the islands do not show real connection with the acini. Oertel [18] and Vincent,[22] however, even express the opinion that there is no morphologic proof at all to separate the islands, as organs with internal secretion, from the rest of the pancreas.

* Received for publication September 13, 1926.

It seems surprising to find such wide divergencies of opinion upon a simple morphologic question. If we inquire into the possible causes of the disagreement among the notable authors, we think that two factors may account for it, *viz.*, inadequate human material and insufficient technic.

In regard to the first factor, it is well known that the pancreas very rapidly undergoes severe postmortem changes, which fact seriously interferes with exact histologic studies. However, it is most difficult to secure human pancreas promptly after death because of institutional regulations. The second factor seems of far greater importance and must be discussed at some length. It is recognized that the pancreas readily shrinks and becomes brittle after routine paraffin preparation and that thin sections are not so easily obtained. The difficulty becomes serious if one intends to prepare serial sections, which is imperative in a question of morphologic relations between various structures. That such difficulties did actually interfere may be inferred from the fact that some of the authors do not illustrate their observations or do so only by inadequate drawings. It seems, however, that if photomicrographs are not available, camera lucida drawings should be presented to support the morphologic conclusions if they are to receive serious consideration.

Of the greatest importance in our opinion is the choice of the proper staining method, a point which has been neglected, with few exceptions. The question of the relationship between islands of Langerhans and surrounding structures centers in the question of the presence or absence of limiting fibers. Therefore, the solution of the problem rests upon the most exact method for their demonstration and on the examination of sufficiently long series of sections. Only the silver impregnation is capable of bringing out the whole of the finest tissue fibers. The claim can be made that our question can only be settled by examination of a considerable number of specimens in thin serial sections prepared with silver impregnation. Since this requirement has not been complied with, we decided to study anew the question of the relation between islands of Langerhans and surrounding pancreatic structures.

MATERIAL AND METHODS

The pancreases of forty-six individuals ranging in age from 3 days to 76 years were examined; 22 adults from 20 to 76 years, 13 children from 1 to 13 years and 11 babies from 3 days to 11 months. In all cases the tissue was removed from the body within five hours after death. In one-third of the cases sections from head, body and tail of the pancreas were examined, in the other two-thirds only one region was cut.

The tissue was fixed in 20 per cent formalin (8 per cent formaldehyde), Helly-Maximow and Lane's solution. The blocks were embedded in paraffin and cut in serial sections 3 to 5 microns in thickness, series averaging 30 to 40 sections. The series were stained by the Bielschowsky-Maresch silver impregnation slightly modified by the author. The impregnation was followed by hematoxylin-eosin stain. For comparison and detailed studies of the cellular granulations, hematoxylin-eosin, Bensley's neutral gentian and the azure B stain developed by MacNeal and the author were employed.

Silver impregnation method on paraffin sections, modified by the author. Fix pieces 3 mm. thick in 20 per cent formalin two to three days; wash thoroughly in running water over night; embed in paraffin.

1. Place the deparaffinized slide in 2 per cent silver nitrate solution in dark staining jar at 37 C for 24 hours or more until section is pale yellow.
2. Wash in distilled water one second.
3. Transfer the slide immediately into the ammoniated basic silver solution in dark staining jar and leave it for one hour at room temperature.

This solution must be freshly prepared each time, as follows: To 20 cc. of 1 per cent silver nitrate solution add 1 drop of 40 per cent sodium hydroxide. Let the test tube stand one minute without shaking and then add 2 to 3 drops of strong ammonia (28 per cent). Shake the test tube slowly. If the solution is not clear, add one more drop of ammonia. The precipitate should not be dissolved entirely; however, the supernatant solution must remain clear. Heat the solution to about 50 C. Filter into dark staining jar. The slide must be placed in this solution while it is still warm. (To obtain a constant satisfactory result, the ammonia solution should be kept in a bottle with a paraffined glass stopper.)

4. Wash in distilled water one second and immediately put the slide into 2 per cent formalin, 1 to 3 minutes.
5. Wash in water and stain with hematoxylin-eosin.

Note: As the precipitate after impregnation usually does not occur in the sections, it is not necessary to use gold chloride solution.

The description of the histologic findings was made from the silver preparation.

HISTOLOGIC OBSERVATIONS

Before entering upon the questionable relation between islands and surrounding pancreatic structures we have to make mention of the arrangement of the fibrillar framework within the pancreas, as we have observed it with the silver impregnation.

Generally the acinus is surrounded by fine black fibers which form a latticed network, as can be recognized in serial sections (Fig. 9). These fibers serve as the basement membrane of the acinar epithelium and as the supporting framework of the blood capillaries. Between acinus and island, fibers of the same caliber are seen (Figs. 3 and 15); therefore they can be recognized as the basement membrane of acini. Branches of these fibers, however, extend into the island and circumvene the insular capillaries. Similar fibers can be found around the excretory ducts.

According to our observations the islands in the specimens examined can be divided into three types: A, islands continuous with acini; B, islands directly continuous with ducts; and C, islands strictly separated from the surrounding tissue.

Type A. Islands connected with acini without intervening fibers. This type was found in every specimen of our series though in varying frequency. A smaller or larger segment of the insular circumference may be continuous with the surrounding acinar cell groups. The following cases may illustrate that type.

Case 17.* Male. 52 years old. Diagnosis: Bronchopneumonia; arteriosclerosis; chronic endocarditis.

A medium-sized island was followed in nineteen serial sections 5 microns thick. In three sections the island is sharply separated from the surrounding acini by their limiting basement membranes. In the other sixteen sections, however, the insular cells are in direct contact with surrounding acini.

Fig. 1A shows at the left side of the island an acinus separated from the insular cells by fine fibers. In the next section, however, Fig. 1B, the limiting fibers are interrupted and the acinar cells blend with the insular cells. Similar conditions are seen on the opposite (right) side of the island. The pictures present the one extreme in which the island is almost completely separated from the surrounding acini. Only serial sections and the demonstration of the small

* Case number refers to our protocol.

breaks within the limiting fibers permit a correct interpretation. The majority of the islands in this case show a similar structure but with considerable variation in the extent of the continuity between insular and acinar cell groups. However, one finds occasionally a small island which is completely separated from the surrounding structures without any break in the limiting fibers in same section. (See left side of Fig. 1A and 1B.)

Case 13. Female, 9 years. Diagnosis: Tumor cerebri; lobar pneumonia.

Here islands are found which showed no limiting fibers at all around the insular circumference, the insular cells freely blending with the acinar cell groups (Fig. 2). This picture represents the other extreme of Type A.

Case 82. Male, 27 years. Diagnosis: Postoperative shock (tenorrhaphy of left hallux).

Fig. 3 shows the one extreme in which the island is connected with acinar cell groups in moderately wide segments; these pictures have been found most frequently.

Case 55. Male, 3 months old. Diagnosis: Adrenal tumor.

Fig. 4 shows an identical picture.

Islands of Type A within the limits of the extremes represented in Figs. 1, 2 and 3 were found in great numbers in all cases examined. In fact it is the type most frequently observed in our material.

Type B. Islands connected with ducts. Here we may distinguish two sub-groups: (*a*) The area of continuity between islands and ducts is very narrow; (*b*) the duct and island are in broad communication.

Case 13. Female, 9 years. Diagnosis: Tumor cerebri; lobar pneumonia.

At the periphery of a lobule a medium-sized, oval island is found in connection with a duct. Followed in serial sections one can see that the epithelial lining of the duct passes into the insular cell groups at one end. There are no limiting fibers found throughout the series (Fig. 5). Other similar islands are found at the periphery of the lobules. There is no evidence of pathology of the pancreas.

Case 72. Male, 3 years. Diagnosis: Pneumonia; otitis media.

A very small island is connected with the epithelium of a dilated duct (Fig. 6).

Case 67. Male, 11 months. Diagnosis: Bronchopneumonia.

Fig. 7 shows a branch of an interlobular duct leading directly into an island. Note the absence of limiting fibers at the area of contact.

Similar conditions have been frequently observed in the pancreas of infants.

Case 73. Male, 71 years. Diagnosis: Hemorrhagia cerebri; cirrhosis of the liver; arteriosclerosis.

Fig. 8 shows a duct with two islands attached. Whereas one side shows intact epithelial lining separated from the island by several layers of fibers, the opposite side shows a wide break in the limiting fibers and communication with the insular cells. The next section shows limiting fibers on both sides.

Similar observations were made in three further cases.

Type C. Islands entirely separated from the surrounding pancreatic structure.

Case 64. Female, 32 years. Diagnosis: Postoperative peritonitis.

In this case a considerable number of islands appear completely separated from the surrounding acini by fine fibers. One of these islands was studied in twenty-two serial sections, 4 microns thick. It is round and in the periphery of a lobule attached to an interlobular excretory duct. No break in the limiting fibers is observed as evidenced in the picture of the series (Fig. 9). In this case there is proliferation of the fat tissue surrounding the pancreas which even grows between the lobules (lipomatosis). There is, however, no evidence of previous inflammation, although the fibers are slightly thicker than usual, most probably due to the slight atrophy of the acini. In this case three medium-sized and three very small islands with identical structure are found; the majority of the islands, however, show continuity with the acini, though to a rather small extent of the circumference (Type A).

Case 8. Male, 34 years. Diagnosis: Peritonitis after perforated ulcer of duodenum.

Two adjoining islands within a lobule are entirely separated from the surrounding acini by fine fibers. No inflammatory changes or increased connective tissue are found within the pancreas (Fig. 10).

Case 34. Male, 1 year. Diagnosis: Bronchopneumonia.

Similar islands are found and one followed in twenty-two serial sections, 4 microns thick. The island is completely separated from the surrounding structures (Fig. 11). No evidence of pathologic changes in the pancreas.

Case 80. Male, 59 years. Diagnosis: Pneumonia.

The pancreas shows marked lipomatosis. Within the fat tissue a great number of isolated islands are found. In serial sections they prove to be entirely separated from the surrounding tissue (Fig. 12).

Case 28. Female, 46 years. Diagnosis: Embolism of pulmonary artery; status post-suspensionem uteri.

Numerous islands are entirely separated by very thick connective tissue fibers from the surrounding acini (Fig. 8). The marked general fibrosis of the septa between the lobules is conclusive evidence of previous inflammation (Fig. 13).

This type of island is found only after considerable search.

Among these three types of islands, Type A is the most frequent, while the others are found only after considerable search. The three different types occasionally occur in one and the same pancreas in fairly identical proportions, even in one section. Generally, however, one type predominates. This fact is clearly demonstrated in Figs. 14 and 15. The former shows large, irregularly outlined islands directly connected with acini. The latter, however, contains round or oval islands, for the most part strictly separated from the acini. Occasional islands show direct contact with the acini but only in a small segment of the circumference.

Islands of Type B are connected with interlobular or intralobular pancreatic ducts. However, it seems self-evident that the islands connected with acini are *eo ipso* continuous with the terminal ducts. That feature, however, is not clearly recognizable because the terminal ducts are mostly collapsed.

In regard to size and shape we find great variations among the islands. The distribution varies considerably in different cases. In four cases we found groups of numerous islands within the head of the pancreas, while the other portions showed a fairly even distribution.

COMMENT

According to the relation of islands to the surrounding pancreatic structures we have found the usual occurrence of three types in the human pancreas. Our observations differ from the results of nearly all former investigators who described the occurrence of only one type of island. Our studies corroborate Laguesse,[14] however, in his opinion that the islands are continuous with the acini as well as the

ducts and prove the occasional occurrence of strictly separated islands, a fact which was only surmised by him.

We attribute the difference in our findings to the method employed and the number of cases examined. We have based our conclusions exclusively on the actual findings in the postfetal pancreas.

Our observations have conclusively shown that the type of islands which are in direct contact with the surrounding acini is the most frequent one found in our material. That such a frequency is not the result of pathologic changes in the pancreas is evidenced by the fact that our observations include a great number of newborn and young children and that identical conditions were found by us in healthy animals such as rabbits, guinea-pigs, hens and pigeons. We may even add that children show a distinct prevalence of this type. This fact also argues against the contention of Weichselbaum and Kyrle,[23] Helly,[7] Opie,[17] Nakamura [16] and others, who refuse to accept the occurrence of connections between islands and acini, because such connections occur only in a very early period of fetal life and disappear later.

Notwithstanding the fact that the histologic methods employed by the above-mentioned authors are open to argument, the predominance of islands of Type A in the pancreas of newborn babies and children indicates that the structure of the pancreas undergoes further changes in the early postfetal period.

Islands connected with ducts range second in our material in regard to their frequency. Weichselbaum and Kyrle and Helly have described the rare occurrence of that insular type in normal pancreas, whereas Opie,[17] Pearce,[19] Karakascheff,[11] Seyfarth,[20] Nakamura[16] and others have demonstrated connection between islands and ducts in cases of congenital syphilis. Our observations are more in accord with those of Laguesse [14] who maintained that such an occurrence is rather the rule than an exception. Similar opinion is held by Bensley [1] for the pancreas of the guinea-pig.

The existence of islands entirely separated from the surrounding pancreatic tissue could be demonstrated beyond doubt in five specimens of normal pancreas. Whereas Weichselbaum and Kyrle and Helly and others maintain that this type of island is commonly found, Herxheimer [10] and Seyfarth [20] believe that it only occurs in pathologic organs, as in fibrosis. Our observations, while contradicting the latter view, fail to confirm the former statement, because

the separated type of island was the least frequent in our material although its occurrence cannot be denied. However, we have frequently seen islands apparently completely separated from the surrounding structures in which only the study of complete serial sections revealed a minute connection with an acinus. Such pictures suggest the possibility that islands of this type might easily become completely separated because there is no doubt that such completely separated islands are very frequent in acquired pathologic conditions of the pancreas, as fibrosis or lipomatosis.

The question as to the existence of a fibrous capsule of the island has been frequently discussed in the literature without definite agreement. Since we conceive the limiting fibers around the islands as the basement membrane of the acini it is self-evident that the occurrence of a thick collaginous fibrous capsule is conclusive proof of a pathologic condition of the pancreas.

Summary and Conclusions

1. The pancreases of forty-six individuals ranging in age from 3 days to 76 years were studied in serial sections stained with silver impregnation in order to determine the relation of the islands of Langerhans to the surrounding pancreatic structures.

2. Three types of islands were recognized in the normal pancreas: A, islands connected with surrounding acini, found in every case; B, islands connected with interlobular and intralobular ducts; and C, islands strictly separated from the surrounding structures. The classification of the types was made in the order of their frequency.

3. The islands have no fibrous capsule of their own but a more or less complete separation is produced by the basement membrane of the acini or ducts or by the interlobular connective tissue.

4. There are wide variations in size, shape and relation of the islands to the surrounding pancreatic structures. Such differences are present not only in the same organ but even in the same sections. Therefore, it is improper to speak of one regular type of the island of Langerhans in respect to this relation.

REFERENCES

1. Bensley, R. R. Studies on the pancreas of guinea-pigs. *Am. J. Anat.*, 1911–12, xii, 297.
2. Dale, H. H. On the islet of Langerhans in the pancreas. *Phil. Tr. Roy. Soc. London*, 1905, cxcvii, 25.
3. De Witt, L. M. Morphology and physiology of areas of Langerhans in some vertebrates. *J. Exper. Med.*, 1906, vii, 193.
4. Flint, J. M. Das Bindegewebe der Speicheldrüsen und des Pankreas, etc., *Arch. f. Anat. u. Entwickl.*, 1903, 61.
5. Fahr, Th. Diabetes Studien. *Virchows Arch. f. path. Anat.*, 1914, ccv, 247.
6. Gellè. Über die Entwicklung der Langerhansschen Inseln bei der Wirbeltieren in normaler, experimenteller und pathologischer Hinsicht. *Ergebn. d. Anat. u. Entwickl.*, 1911, xx, 1042.
7. Helly, K. Studien über Langerhansschen Inseln. *Arch. f. mikro. Anat.*, 1905, lxvii, 124.
8. v. Hansemann. Über die Structur und das Wesen der Gefässinseln des Pankreas. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, iv, 187.
9. Herxheimer, G. Weitere Untersuchungen am Pankreas von Diabetikern. *Verhandl. d. deutsch. path. Gesellsch.*, 1905, 263.
10. Herxheimer, G. Zur Pathologie des Pankreas. *Verhandl. d. deutsch. path. Gesellsch.*, 1909, 276.
11. Karakascheff, K. I. Neue Beiträge zum Verhalten der Langerhansschen Inseln bei Diabetes mellitus und zu ihrer Entwicklung. *Deutsch. Arch. f. klin. Med.*, 1906, lxxxvii, 291
12. Küster, H. Zur Entwicklungsgeschichte der Langerhansschen Inseln beim menschlichen Embryo. *Arch. f. mikro. Anat.*, 1904, lxiv, 158.
13. Koch, K. Über die Bedeutung der Langerhansschen Inseln in menschlichen Pankreas. *Virchows Arch. f. path. Anat.*, 1913, ccxi, 321.
14. Laguesse, E. Sur l'evolution des ilots endocrines dans le pancreas de l'homme adulte. *Arch. d'Anat. micro.*, 1909, xi, 1.
15. Lane, M. A. The cytological characters of the areas of Langerhans. *Am. J. Anat.*, 1907–08, vii, 409.
16. Nakamura, N. Untersuchungen über das Pankreas bei Föten, Neugeborenen, Kinder und im Pubertätsalter. *Virchows Arch. f. path. Anat.*, 1924, ccliii, 286.
17. Opie, E. L. On the histology of the islands of Langerhans of the pancreas. *Bull. Johns Hopkins Hosp.*, 1900, xi, 205.
18. Oertel, H. The pancreas and diabetic metabolism. *Lancet*, 1924, i, 695.
19. Pearce, R. M. The development of the islands of Langerhans in the human embryo. *Am. J. Anat.*, 1902–03, ii, 445.
20. Seyfarth, C. Neue Beiträge zur Kenntniss der Langerhansschen Inseln im menschlichen Pankreas und ihrer Beziehung zum Diabetes mellitus. Jena, 1920.
21. Vincent, S., and Thompson, F. D. On the relation between the islets of Langerhans and the zymogenous tubules of the pancreas. *Internat. Monatschr. f. Anat. u. Physiol.*, 1907, xxiv, 61.

22. Vincent, S. The relationship between the islets of Langerhans and the zymogenous tissue of the pancreas. *Lancet*, 1924, i, 947.

23. Weichselbaum, A., and Kyrle, J. Über das Verhalten der Langerhansschen Inseln in fötalen und postfötalen Leben. *Arch. f. mikro. Anat.*, 1909, lxxiv, 223.

DESCRIPTION OF PLATES

All preparations illustrated except Figs. 12 and 13 were made by the silver impregnation method.

PLATE I

FIG. 1. An oval island shows direct connection with acinar cell groups in a small segment of the circumference. See the description on p. 4. A small round island which has been followed in 14 serial sections is entirely separated from the acini.

FIG. 2. An island connected with the acinar cell groups directly in the greater part of its circumference. There are no limiting fibers at all around the island.

PLATE 2

FIG. 3. An island connected with the acini in a moderate portion of its circumference.

FIG. 4. Islands not limited from the acini.

PLATE 3

FIG. 5. An island connected with a duct.

FIG. 6. An island connected with a duct.

PLATE 4

FIG. 7. Undifferentiated insular cell groups connected with a branch of a duct.

FIG. 8. An island connected with the epithelial lining of a duct.

PLATE 5

FIG. 9. An island everywhere strictly separated from the rest of the pancreatic tissue by fine fibers. A complete series.

PLATE 6

FIG. 10. Islands sharply separated by fine fibers.

FIG. 11. One island (in the center) is sharply separated; the other islands show direct connection with acini.

PLATE 7

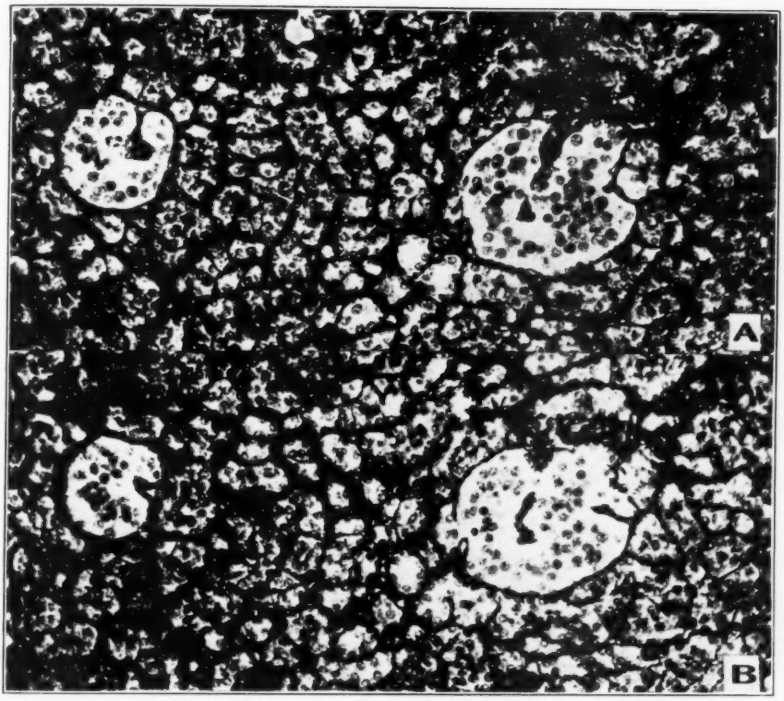
FIG. 12. The islands which remain completely separated in the fat tissue (lipomatosis) (followed in serial sections). Hematoxylin and eosin.

FIG. 13. Islands surrounded by thick connective tissue fibers. Hematoxylin and eosin.

PLATE 8

FIG. 14. Pancreas with irregular form of the islands connected with acini.

FIG. 15. Pancreas with rather round islands, most of them separated from the rest of the pancreatic tissue, while few islands are connected with the acini.



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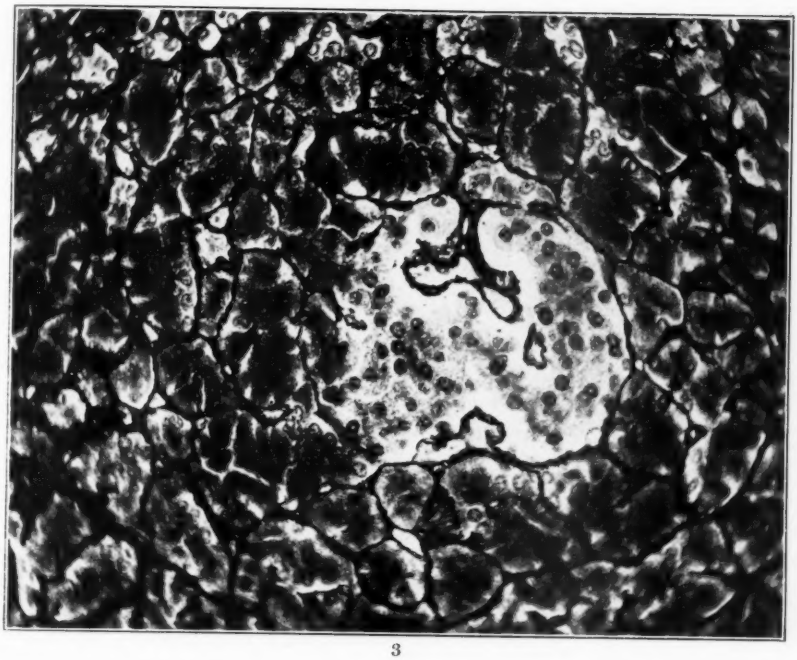
Otani

Islands of Langerhans



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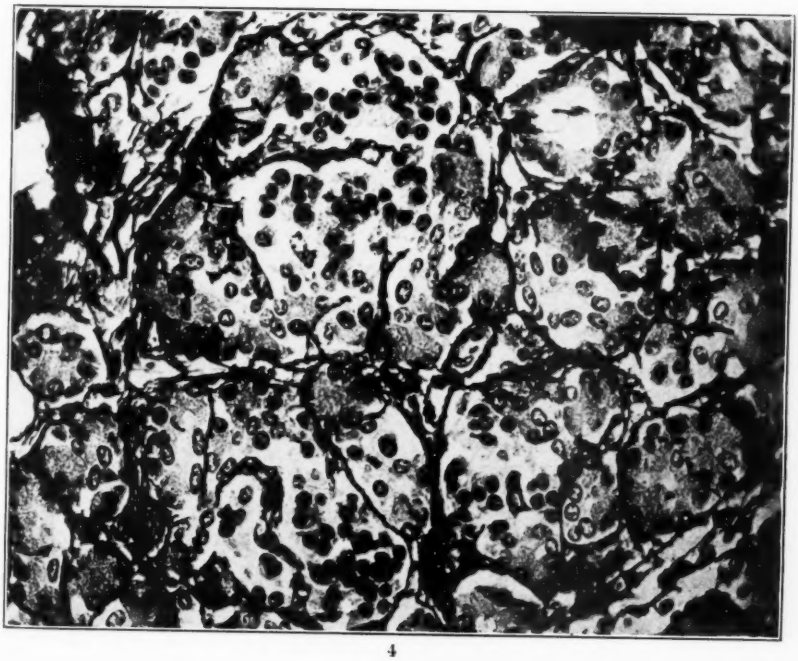

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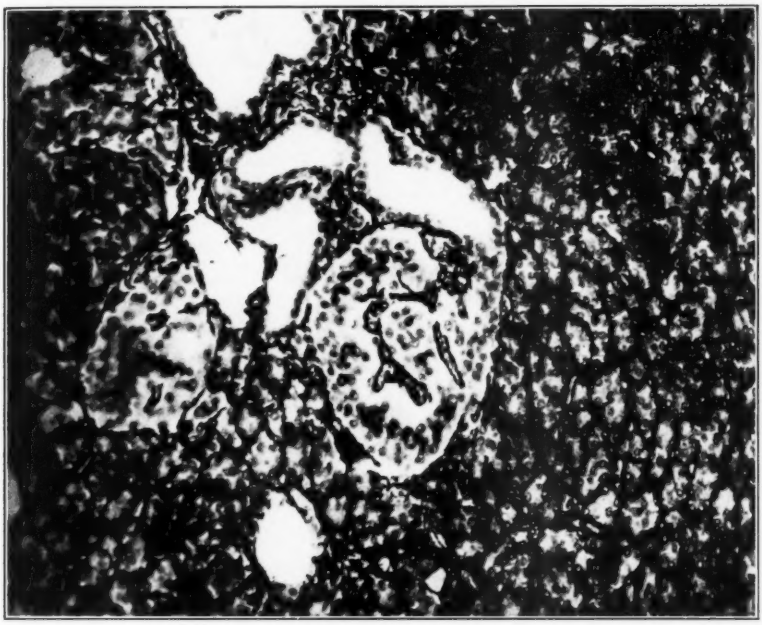
Islands of Langerhans



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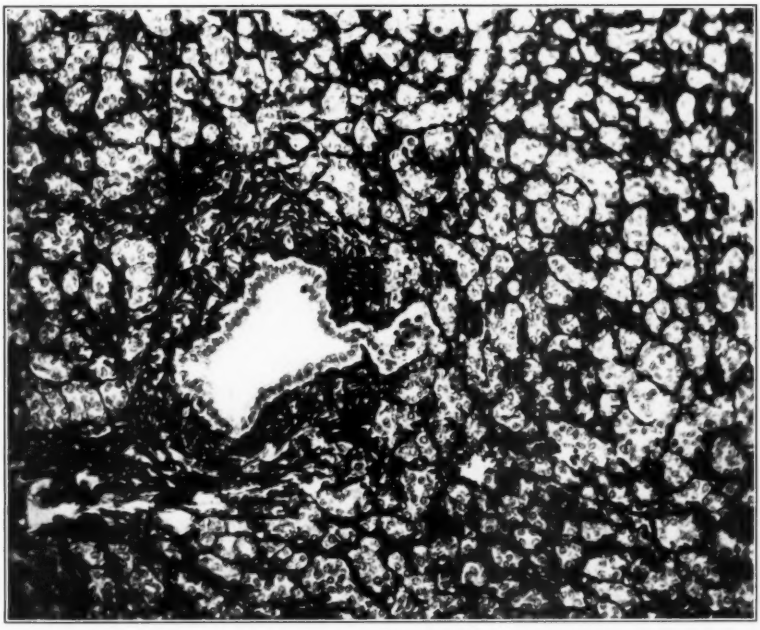

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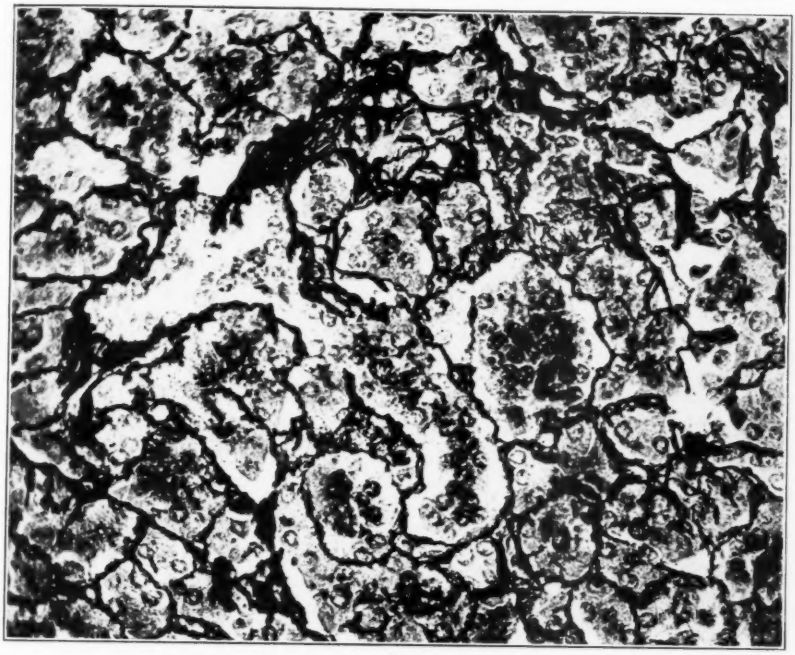

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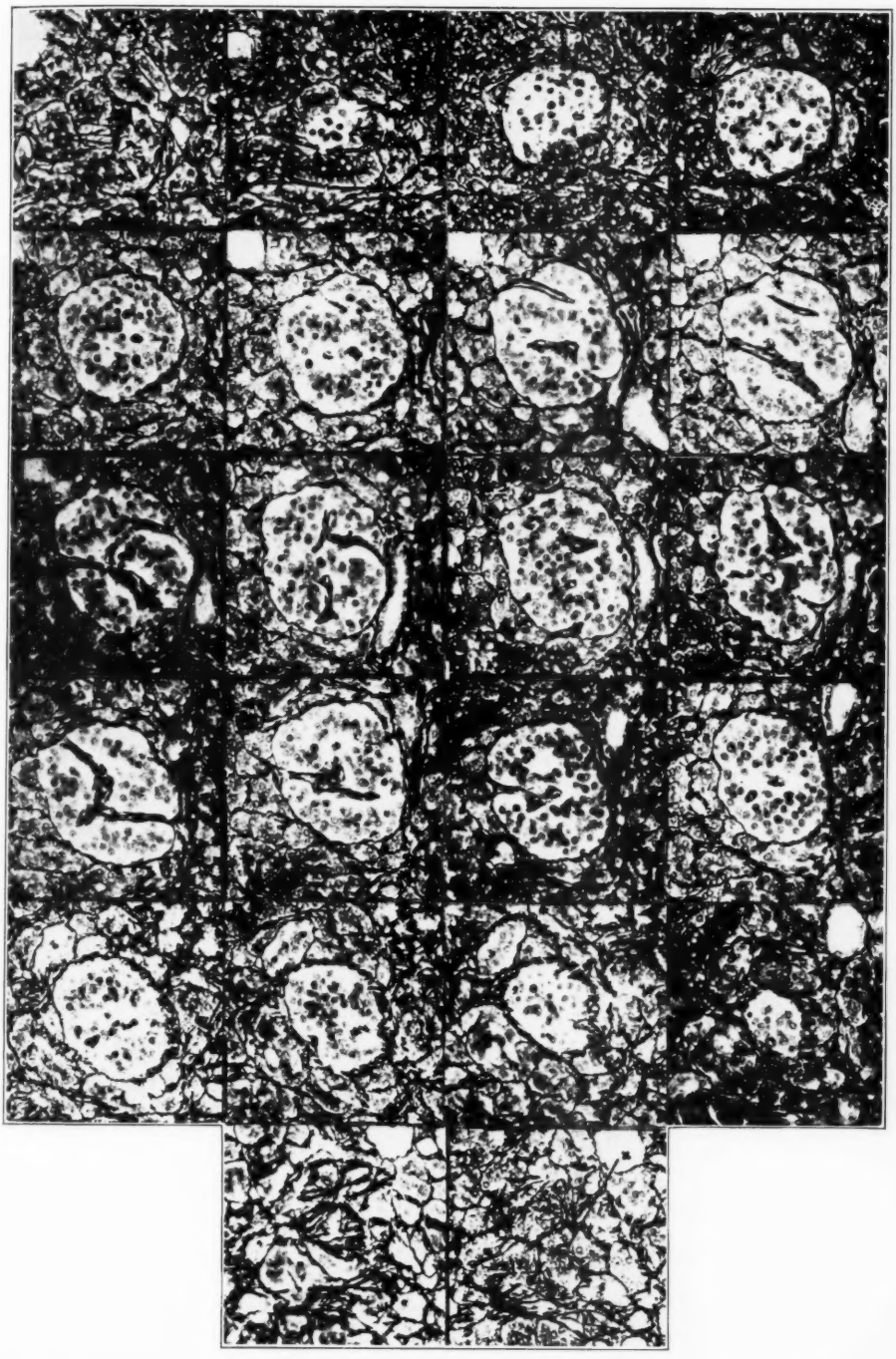
Otani

Islands of Langerhans



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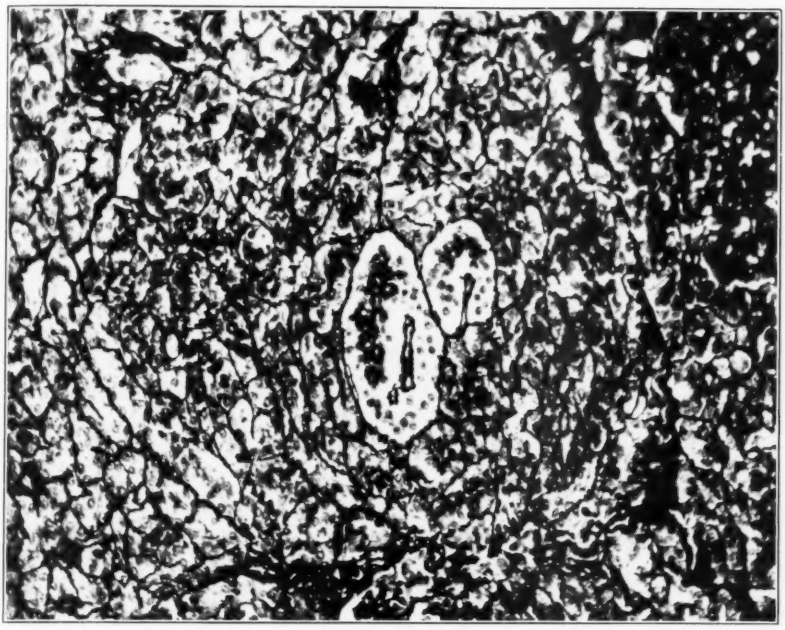
Otani Islands of Langerhans



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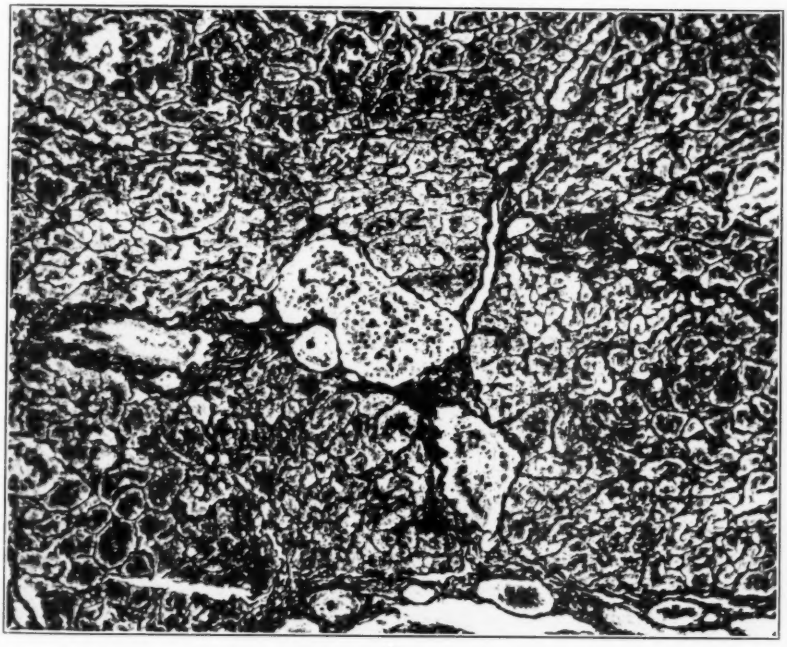

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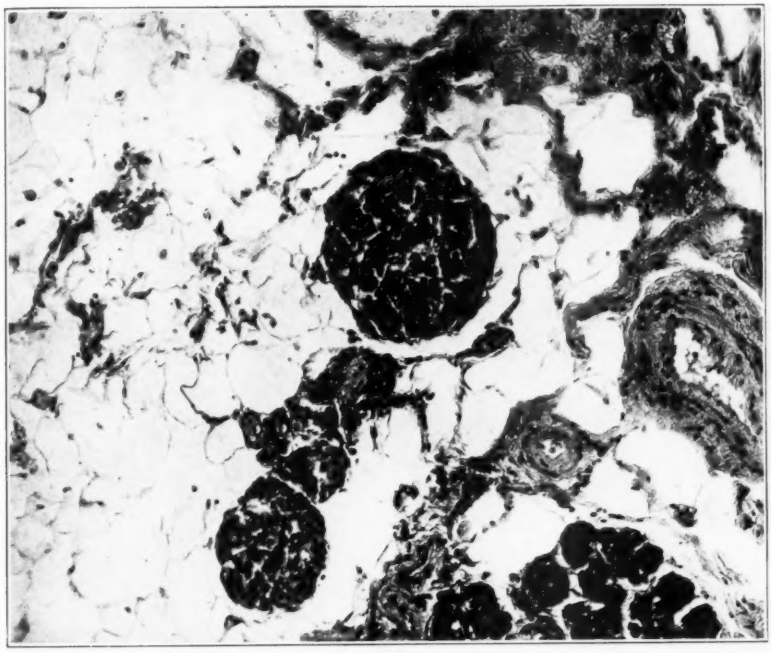
Otani Islands of Langerhans



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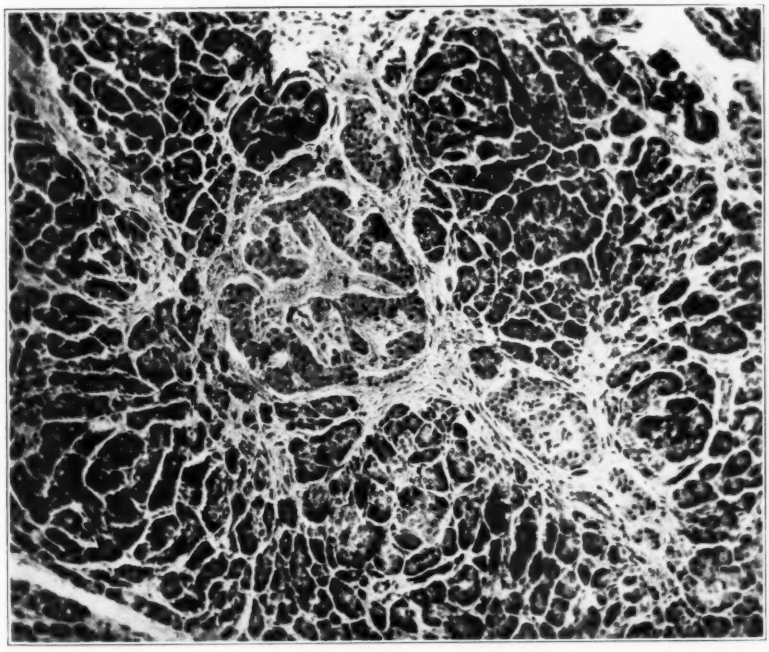

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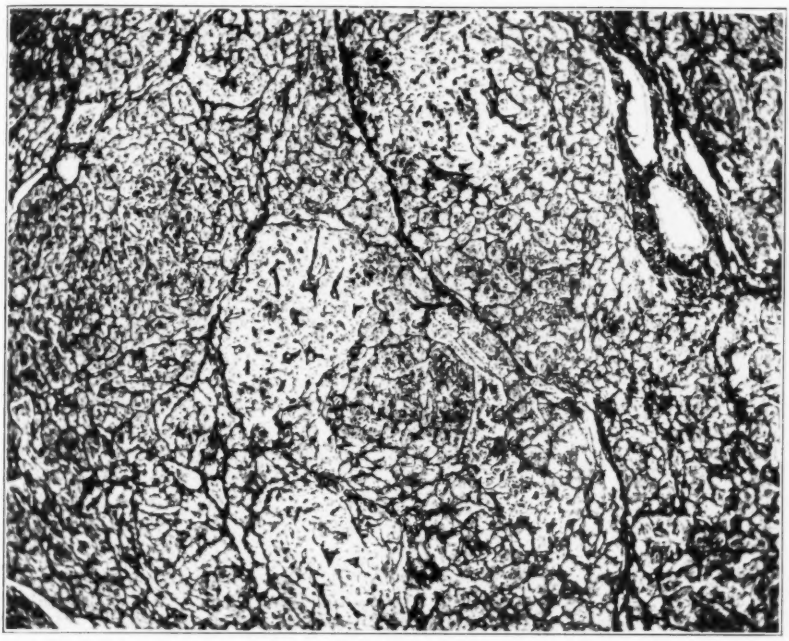
Otani

Islands of Langerhans



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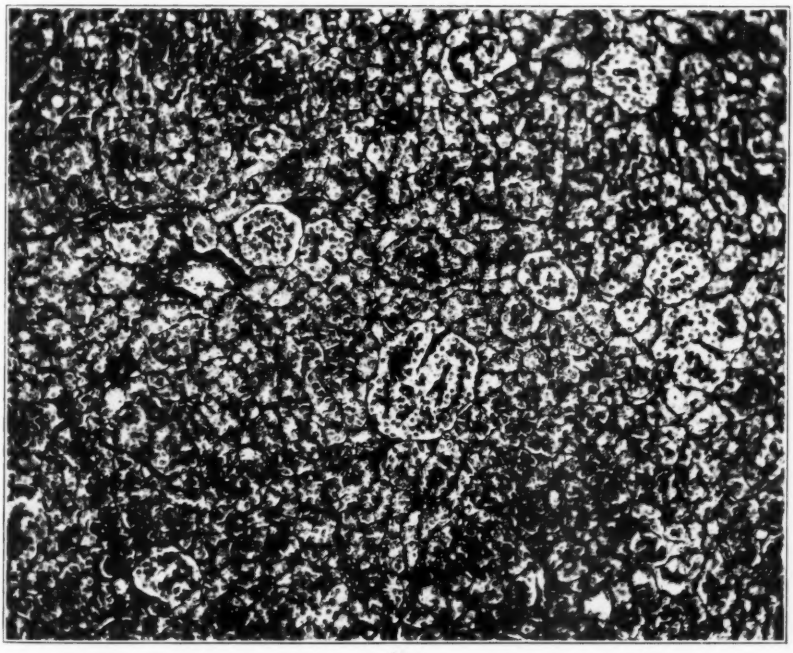


15

Otani Islands of Langerhans

THE ORGANIZATION OF EXPERIMENTAL ADRENAL CELL EMBOLI IN THE LUNGS *

J. P. SIMONDS

(From the Department of Pathology, Northwestern University Medical School, Chicago, Ill.)

In the course of experiments intended to produce a cytolytic serum for adrenal cells, a somewhat coarse emulsion of dog adrenal tissue was injected into the ear vein in a number of rabbits. As was expected small emboli composed of groups of adrenal cells lodged in the lungs. These emboli did not of themselves cause death of the animals, although some of them died very quickly after the third or fourth intravenous injection just as they sometimes do in any attempt to produce antibodies to other antigens by this method. From these animals a series of lungs was obtained containing emboli from a few minutes to two weeks old.

The organization of these adrenal cell emboli was characterized by a reaction so different from that of ordinary emboli that it has seemed worth while to present this brief report.

In the earlier stages numerous small blood vessels in the lungs were found plugged with finely granular material with a few pyknotic nuclei and vague adrenal cell outlines. In some cases the adrenals had been preserved in glycerine for several days before being ground up or injected into the rabbits. This accounts for the partially necrotic condition of the adrenal cells found in the pulmonary vessels of those animals that died immediately after the intravenous injection.

On casual low-power examination, those emboli in which the process of organization was well advanced resembled tubercles. The older ones contained one or more giant cells with four to twenty or more nuclei irregularly scattered through the cytoplasm, thus resembling foreign body giant cells rather than typical Langhans cells. The central part of the organizing mass was frequently necrotic but this central area was composed of dead adrenal cells and not of necrotic, newly formed tissue as in a tubercle. The greater number

* Received for publication August 18, 1926.

of elements in this mass, however, were large, oval or spindle-shaped cells with abundant cytoplasm, rather indefinite outlines and round, oval or indented vesicular nuclei, thus resembling the epithelioid cells of a tubercle. Surrounding this pale central mass of epithelioid and giant cells was a zone of densely crowded lymphocytes. In several of the animals eosinophiles were present in great numbers in the outer edges of the lesions and the immediately adjacent lung tissue. In some instances the organizing mass appeared to have become canalized for it showed a small blood-filled channel usually eccentrically located.

Much the same type of change occurred in the process of organization of a similar emulsion of adrenal tissue injected subcutaneously into rabbits. In the latter location, however, an Arthus phenomenon of an especially violent type was produced after two to four subcutaneous injections of emulsion of dog adrenals at intervals of two to three days. This occurred much more constantly and more promptly than after the injection of horse serum. In the subcutaneous tissues, therefore, the process of organization of the adrenal fragments was complicated by the process of necrosis and inflammation incident to Arthus phenomenon. However, even here the same general type of reaction (formation of giant cells, epithelioid cells and accumulation of lymphocytes and sometimes of eosinophiles) could be readily distinguished.

In the organization of thrombi, of emboli originating from thrombi, of infarcts and of transplants of various tissues other than adrenal, epithelioid and giant cells may or may not take part in the process. They are specifically mentioned by Loeb [1] in the organization of transplants of kidney, liver and spleen. They are not referred to by Fleisher [2] and in other papers by Loeb in association with kidney transplants. Loeb does not mention them in relation to transplants of thyroid,[3] skin [1] and corpus luteum.[1] He observed them in the organization of blood clots, being most numerous at places where the clot was especially dense and offered resistance to the progress of the invading fibroblasts. Karsner and Dwyer [4] described them in organizing infarcts of the myocardium.

While epithelioid and giant cells are often present in the processes of organization just mentioned, they do not play such a predominant rôle as in the replacement of the emboli of adrenal cells described above. They have been observed by others in transplants of adrenal

tissue. Poll [5] saw large cells with as many as ten nuclei in adrenal transplants. He thought that they probably originated from adrenal cells because Manasse [6] had described multinuclear cells in "hyperplastic adrenal tumors." Schmieden [7] observed similar cells in adrenal tissue implanted in the kidney and believed that some of them were derived from the transplanted cells and that others were true foreign body giant cells. Stoerk and von Haberer [8] grafted bits of adrenal with vessel attached beneath the capsule of the kidney and found giant cells both in the renal tissue and in the transplant. They also mention cholesterol slits surrounded by foreign body giant cells.

The reaction, above described, of the lungs of these rabbits to small masses of adrenal cells lodged in their pulmonary vessels is distinctly of the foreign body type. This form of reaction is believed to be due to the chemical nature of the emboli. The formation of foreign body giant cells about crystals of cholesterol in the tissues is well known. Wells [9] found that 36.3 per cent of the dried adrenal gland consisted of ether-soluble material of which 20.6 per cent was cholesterol and 33 per cent phospholipins. Long [10] has stated that approximately 40 per cent of the dry weight of the tubercle bacillus consists of fat-like substances (lipins) but it apparently contains no cholesterol. There are, therefore, certain relationships between the chemical composition of the adrenal emboli and that of tubercle bacilli. This may be the basis for the similarities between tubercles and the nodules produced by the organization of the adrenal emboli in the lungs of these rabbits.

Summary

Certain resemblances are pointed out between the histologic tubercle and nodules produced in the lungs of rabbits by the organization of experimental adrenal cell emboli. This peculiar type of reaction is believed to be due to the high content of lipoid substances, especially cholesterol, contained in the adrenal cells.

REFERENCES

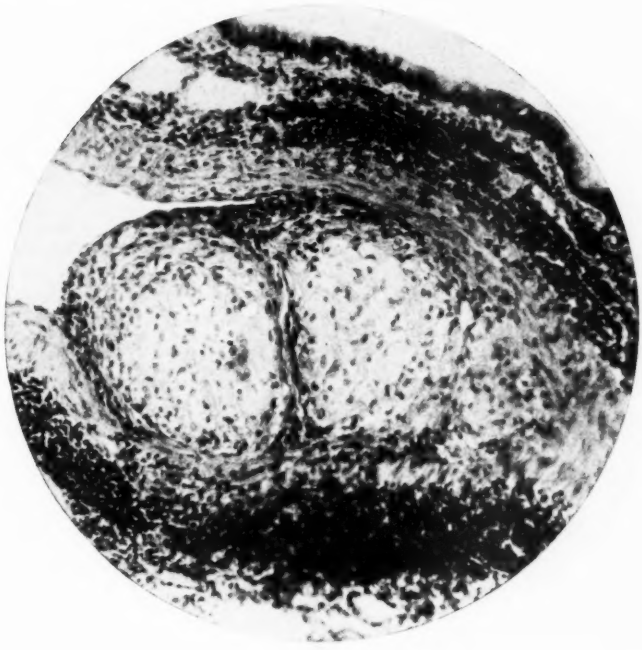
1. Loeb, L. *J. Med. Res.*, 1917, xxxvii, 229; 1918, xxxix, 189.
2. Fleisher, M. S. *J. Med. Res.*, 1917, xxxvii, 483.
3. Loeb, L. *J. Med. Res.*, 1920, xli, 305.
4. Karsner, H. T., and Dwyer, J. E. *J. Med. Res.*, 1916, xxxiv, 21.

5. Poll, H. *Arch. f. mikro. Anat.*, 1899, liv, 440.
Centralb. f. Physiol., 1898, xii, 321.
6. Manasse. *Arch. f. Path. Anat. u. Physiol.*, 1895, cxxxiii, 21.
7. Schmieden, V. *Deut. Ztschr. f. klin. Chir.*, 1903, lxx, 453.
8. Stoerk, O., and von Haberer, H. *Arch. f. klin. Chir.*, 1908, lxxxvii, 893.
9. Wells, H. G. *J. Med. Res.*, 1908, xvii, 461.
10. Long, E. R. Chemistry of Tuberculosis by Wells, De Witt and Long. 1923, 35.

DESCRIPTION OF PLATES

PLATE 9

FIG. 1. Organization of adrenal cell embolus. Diagonal section. × 170.
FIG. 2. Organization of adrenal embolus. Transverse section. Cholesterol slits not in focus, to right of giant cell. Eosinophiles above giant cell. × 350.



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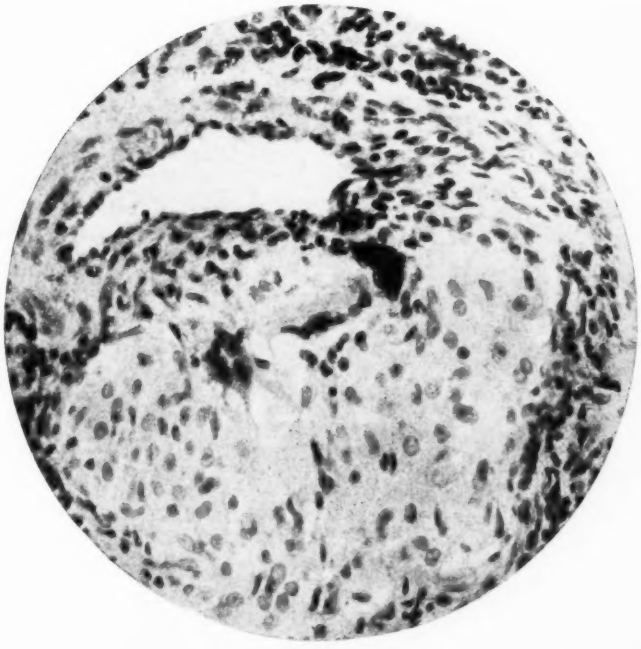

2

Simonds Organization of Adrenal Cell Emboli

A QUANTITATIVE STUDY OF THE HYPOPHYSIS OF THE HUMAN ANENCEPHALIC FETUS *

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INTRODUCTION

The question of the presence or the absence of the hypophysis in the anencephalic fetus has been of special significance in connection with the subject of the function and interrelationship of the ductless glands. Some have even attributed this abnormality to a disturbance of the endocrine system. Previous to 1921 the general belief was that an hypophysis was frequently absent in anencephalics and when present was usually described as being different from the normal gland. No quantitative methods have been applied, consequently the literature is based on qualitative observations only.

It is the purpose of this investigation to apply quantitative methods to these abnormal specimens and when the organ is present to compare it with that of the normal. In order to bring the subject to its present status the principal contributions are summarized below.

REVIEW OF THE LITERATURE

Ballantyne [1] has described forty-five anencephalic fetuses and states that "the hypophysis rarely occurs." Schwalbe [2] says the hypophysis is sometimes present and in a large number of cases there is accessory hypophyseal tissue. Haberfeld [3] examined three cases of anencephaly and reported an hypophysis as present in each. In one instance a pars nervosa was found in the upper part of the craniopharyngeal canal. The glandular portions, in this instance, extended through the canal and only a small polyp was present at either end. Brown [4] examined five specimens of anencephalic fetuses and failed to find the hypophysis. He concluded, therefore, that the condition of apituitarism was normal for anencephaly.

Mauksch [5] found an hypophysis in nine cases of this monstrosity. In five of these, he described a pharyngeal hypophysis with a par-

* Received for publication September 28, 1926.

tially or totally patent craniopharyngeal canal which contained blood vessels and hypophyseal tissue. All three lobes were present in only two of the nine glands. When there was no posterior lobe (pars nervosa), he did not observe a pars intermedia and thus considered the hypophysis in such cases to consist of pars anterior only. In one instance he found the upper surface of the connective tissue capsule to contain a small neuroglial structure which was in contact neither with the brain tissue nor with the hypophysis. There was no intermediate glandular portion discernible. In two of the specimens the pars nervosa was found to be a small basophilic structure of neuroglial elements located on a concavity of the glandular part. He noticed a pars intermedia in contact with the latter but thought it appeared abnormal and incomplete. He found the pars anterior to be very vascular and the parenchyma in the form of cords surrounded by sinuses filled with blood.

Kohn [6] verified the report of Mauksch [5] as to the occurrence of the hypophysis. He investigated eleven cases of anencephaly and found an hypophysis in each. He likewise found the gland to be abnormal in structure. In only three of the specimens did he find a pars nervosa. He concluded that the pars intermedia and pars nervosa as well as the pars tuberalis are usually lacking. If the pars nervosa was not present, he thought the intermediate lobe was also absent. He reported that the cells of the pars anterior were normally differentiated. In addition he described a cell which he thought occurred but seldom in the normal fetal hypophysis. According to him, this latter mentioned type was present in large numbers in the anencephalic hypophysis. Such a cell type appeared to be larger than the chromophile cells and stained slightly basophilic or neutrophilic with Mallory's triple connective tissue stain.

Wrete [7] found a hypophysis to be present in a case of encephalomyeloschisis totalis of an embryo of the seventh week.

Material and Methods

The material used in this study consisted of thirty-two anencephalic fetuses which were obtained from the Department of Pathology, University of Minnesota. Most of the material had been previously preserved in 10 per cent formalin in which it had remained for a year or more in some instances.

Twenty-seven of the thirty-two fetuses were females, the remaining five being males. They ranged in age from about the fifth fetal month to full term. The ages of the fetuses were determined by means of a formula used by Nañagas [8] for conversion of the leg length (distance from the greater trochanter to the base of the heel) of the anencephalic fetus into total body length of a normal fetus. Then by applying to the result the formula of Scammon and Calkins [9] for converting the crown-heel measurement into terms of age in fetal months, the latter was determined. The formula given by Nañagas is as follows:

$$\text{Crown-heel (cm.)} = \frac{1.00}{0.43} \text{ leg length (cm.)} + 0.7$$

The formula developed by Scammon and Calkins gives the age in fetal months or "T" when "L" is the crown-heel measurement in centimeters.

$$T = 2.3 + \frac{2.5\,L}{28} + \frac{L^2}{784}$$

The relative and absolute volumes of the parts of the gland were determined by the paper-weight method as described by Rasmussen and Herrick.[10] For a comparison of the abnormal with the normal gland, data available (Covell [11]) on the hypophyses of sixty-two normal fetuses were used.

The relative amount of blood in the pars anterior of the hypophysis was approximated by projecting areas of sections 10 microns thick of this portion of the gland at a magnification of about 750 times. Five fields were chosen on each of three different levels, two of the fields being near the periphery of the gland, one centrally located and the remaining two intermediate between the periphery and central parts. The vascular areas in each of the fields were outlined with a hard lead pencil. The relative amount of blood in the pars anterior was then ascertained by weighing the paper areas representing the blood and those representing the fibrous and glandular tissue, and dividing the weight of the vascular areas in paper by the total paper-weight.

For the normal gland, a section approximately one-half of the way through the gland was used and five fields were outlined on it. The relative volume of the blood was found to be very nearly iden-

tical with that determined by the use of the three levels and the fifteen fields.

General Description of the Hypophysis

Of the thirty-two specimens of the anencephalic fetus examined, an hypophysis was present in each instance. Such findings are in accordance with the more recent investigations on this monstrosity but in contradiction to the earlier work. It would appear that the earlier work was of a gross nature so that the small amount of hypophyseal tissue which lies on the malformed basis cranii may have been overlooked.

The hypophyseal fossa is usually lacking or at the most only feebly developed. No trace of the clinoid processes or dorsum sella is evident. Occasionally the anterior clinoid processes are slightly developed and so form a narrow fossa for the most anterior portion of the gland. A diaphragma sella is lacking, probably because of the absence of the clinoid processes.

The hypophysis may then be said to lie on a flattened sella turcica covered over by membranes, vascular tissue and in some instances a small amount of brain tissue. Macroscopically it presents a flat triangular shape with the apex directed dorsally. The base of this mass is formed by two lateral spreading portions and the apex of one central mass. Such a shape no doubt is of embryologic significance because it is known that the glandular portion (pars anterior) grows in early fetal life by means of two lateral and one central buds from the ventral side of Rathke's pouch. This form subsequently changes in the course of normal development because the gland soon approximates the shape of the fossa in which it is situated. Macroscopically, the neural elements (pars nervosa) of the gland, when present, appear as an irregularly shaped opaque mass on the dorsoposterior surface of the larger glandular portion. An infundibular stalk is lacking and only a thin cord of membrane remains to attach the gland to the overlying tissues. Microscopically, the hypophysis of the anencephalic fetus presents several striking differences when compared with the gland from the normal fetus. In the first place, the three lobes may not all be present. In only six of the seventeen specimens studied did the hypophysis consist of three lobes. In eleven instances there was no pars nervosa evident. In four of the latter a pars intermedia could be distinguished, while in the remain-

ing seven the pars anterior appeared to comprise the total gland volume.

The extreme vascularity of the gland, particularly of the pars anterior, presents a marked difference from that of the normal. Especially about the periphery of the anterior lobe the sinuses are dilated to such an extent that the parenchyma is reduced to narrow epithelial cords which appear to be surrounded by blood spaces. Toward the center of the pars anterior, the sinuses are usually less dilated with blood.

Trabeculae radiate through the pars anterior and contain numerous blood vessels. Occasionally there is a band of fibrous tissue separating the main bulk of the anterior lobe from the thin epithelial strip which borders the hypophyseal cavity. Likewise the pars intermedia may be separated from the pars nervosa by a thin band of fibrous tissue. The capsule of connective tissue about the gland is well developed and appears to be more extensive than in the normal hypophysis.

Relative and Absolute Volumes of the Gland and Its Parts

A. *Weight of the total gland.* In Table 1 are given the absolute volumes of seventeen hypophyses and their lobes. It is evident that there is considerable individual variation in the total weight of the gland for fetuses of about the same age. The six observations for fetuses of the fifth to the sixth fetal months have a range of 29.3 to 69.6 mg. and an average of 42.3 mg. The range as observed from six specimens of the sixth to the seventh fetal months is 29.2 to 85.6 mg. with an average of 58.5 mg. Four hypophyses from fetuses of the seventh to the eighth fetal months show a range of 35.0 to 73.8 mg. and an average of 55.1 mg. The intervals from the eighth fetal month to birth are represented by only one observation. The age of the latter was calculated as 10.16 fetal months and the hypophysis weight was found to be 113.2 mg.

B. *Weight of the pars anterior.* Considerable variation is likewise evident in the weights of the pars anterior. For the fifth to the sixth months, the range is 29.3 to 65.4 mg. and the average is 40.9 mg. The weights of this lobe in specimens of the sixth to the seventh fetal months show a range of 28.9 to 85.6 mg. with an average of 57.8 mg. From the seventh to the eighth fetal months, the range is 34.6 to

TABLE 1

Absolute Volume of the Gland and its Parts in Seventeen Specimens of Anencephaly

Case number	Sex	Leg length *cm.*	Total body length computed *cm.*	Age in fetal months	Hypophysis weight *mg.*	Pars anterior weight *mg.*	Pars intermedia weight *mg.*	Pars nervosa weight *mg.*
c9	F	10.2	25.34	5.38	38.0	36.6	0.9	0.5
c2	M	10.7	26.51	5.56	41.6	40.8	0.8	...
c10	F	11.0	27.20	5.66	37.3	37.3	...	...
c4–17	F	11.4	28.14	5.81	69.6	65.4	2.5	1.7
c23–184	F	11.7	28.83	5.93	29.3	29.3	...	...
c4	F	11.7	28.83	5.93	38.4	36.1	1.3	1.0
c23–183	F	12.0	29.53	6.05	72.7	69.5	2.3	0.9
c7	F	12.7	31.16	6.32	50.4	50.4	...	...
c1	F	12.9	31.63	6.39	29.2	28.9	0.3	...
c6	M	13.1	32.09	6.47	41.2	40.3	0.9	...
c8	F	13.2	32.33	6.51	85.6	85.6	...	...
c12	F	13.7	33.49	6.72	72.2	72.2	...	...
c15	M	14.5	35.35	7.05	68.9	68.9	...	...
c3	F	14.6	35.58	7.08	35.0	34.6	0.4	...
c5	F	15.5	37.67	7.47	42.9	41.4	1.5	...
c14	F	16.6	40.23	7.95	73.8	71.4	2.0	0.4
c4–18	F	21.2	50.93	10.16	113.2	106.3	1.6	5.3

TABLE 2

Relative Volumes of the Lobes of the Anencephalic Hypophysis

Case number	Age in fetal months	Pars anterior	Pars intermedia	Pars nervosa
		per cent	*per cent*	*per cent*
c9	5.38	96.33	2.36	1.31
c2	5.56	98.08	1.92	...
c4–17	5.81	93.97	3.59	2.44
c4	5.93	94.01	3.39	2.60
c23–183	6.05	95.58	3.14	1.28
c1	6.39	98.97	1.03	...
c6	6.47	97.82	2.18	...
c3	7.08	98.86	1.14	...
c5	7.47	96.50	3.50	...
c14	7.95	96.75	2.71	0.54
c4–18	10.16	93.90	1.41	4.69

71.4 mg. and the average is 54.1 mg. In the one gland of a fetus of 10.16 fetal months the weight of the pars anterior is 106.3 mg.

C. *Weights of the partes intermedia and nervosa.* The absolute volumes of the partes intermedia and nervosa are of very little significance because of the extreme variations. They are, however, of importance for a comparison with the normal weights.

D. *Relative volumes of the parts (lobes).* The relative volumes of the various lobes of the hypophysis are given in Table 2. Because of the limited number of cases and the apparent irregularities in the partes intermedia and nervosa, the relative volumes will be considered without respect to fetal age. The anterior lobe comprises about 93.9 to 100 per cent of the total gland volume. In the eleven instances in which a pars intermedia was present, it is seen to comprise 1.14 to 3.59 per cent of the total gland weight. The pars nervosa which occurs in only six of the seventeen cases varies in relative volume from 0.54 to 4.69 per cent. From the above percentage values it is evident that most of the hypophysis is pars anterior and only a small part is intermediate and posterior lobes. In only one instance does the pars nervosa exceed the pars intermedia in relative as well as absolute volume.

Volumetric Analysis of the Pars Anterior

A. *Relative volume of the blood.* Because of the engorged sinuses of the pars anterior and the prominent trabeculae, it was found necessary to determine the relative amounts of each. This was accomplished by means of the paper-weight method as already described. The results are tabulated in Table 3. The figures for the relative vascularity appear to vary from about 25 to 50 per cent for individual estimations. The average for the fifteen determinations is 38.98 per cent.

B. *Relative volume of the trabeculae.* The relative amounts of trabeculae present in the gland range from about 0.5 per cent to nearly 4 per cent. The average for the series is 1.58 per cent. This figure represents only the larger masses of fibrous tissue in the pars anterior. The finer connective tissue framework is included with the percentage figure for the parenchyma, the average for which is 59.44 per cent of the volume of the pars anterior. The range is approximately 45 to 77 per cent.

It is thus obvious that considerable of the absolute volume of the anterior lobe is due to the presence of blood in the sinuses. From the relative averages cited above, it may be said that approximately two-fifths of this part of the gland is composed of vascular and

TABLE 3

Volumetric Analysis of the Components of the Pars Anterior

Case number	Blood	Trabeculae	Parenchyma and finer fibrous stroma
	per cent	*per cent*	*per cent*
c9	38.18	3.98	57.84
c2	40.37	0.49	59.14
c4–17	43.81	2.02	54.17
c4	37.83	1.12	61.05
c23–183	36.71	2.34	60.95
c7	34.48	1.03	64.49
c1	21.56	1.40	77.04
c6	46.67	0.75	52.58
c8	53.00	2.16	44.84
c12	40.05	0.97	58.98
c15	37.81	1.59	60.60
c3	27.22	1.46	71.32
c5	38.93	1.47	59.60
c14	36.63	2.00	61.37
c4–18	51.46	0.97	47.57
Mean	38.98	1.58	59.44

fibrous stroma. The remaining three-fifths is comprised of parenchyma and finer fibrous stroma. Such an analysis is of value in determining the actual amount of glandular tissue which is present. The large amounts of blood apparently influence the volume of the anencephalic hypophysis to a marked degree.

DISCUSSION

The shape of the anencephalic hypophysis is in contrast to the normally developed fetal hypophysis. Apparently the hypophyseal fossa plays an important rôle in determining the form and dimensions of the normal fetal gland. Since this fossa is lacking or at the most only feebly developed in anencephaly, the hypophysis is flat-

tened and spreading. For this reason it is obvious that the flattened surface might permit the gland to keep its earlier fetal shape as it appears to do.

The gland of the normal fetus is known to consist of three lobes and, in addition to the main body of the gland, a pars tuberalis. Likewise the hypophysis of the anencephalic may be comprised of three lobes but not a pars tuberalis. It has been shown that a pars

TABLE 4

A Comparison of the Observed Average Values for the Weight of the Anencephalic Hypophysis with that of the Normal Fetal Hypophysis According to Age in Fetal Months

Age in fetal months	Number of cases	Weight of the anencephalic hypophysis *mg.*		Number of cases	Weight of the normal fetal hypophysis *mg.*		Differences in weight between anencephalic and normal fetal hypophyses *mg.*
Range		Range	Mean		Range	Mean	
5 to 6	6	29.3–69.6	43.2	8	16.0– 41.3	30.1	+12.2
6 to 7	6	29.2–85.6	58.5	16	30.2– 52.7	42.4	+16.1
7 to 8	4	35.0–73.8	55.2	12	42.4– 62.3	55.5	– 0.3
8 to 9	..	....	...	13	58.2–121.2	81.2	...
9 to 10	..	....	...	12	78.5–130.1	95.6	...
10.16.....	1	113.2	113.2	1	112.6	112.6	+ 0.6

nervosa occurred in only six of the seventeen cases. The anterior lobe may then be said to form the main bulk of the hypophysis. The pars intermedia of this abnormal gland may or may not be present. The residual lumen (hypophyseal cavity) likewise is not always in evidence. The lobes of the hypophysis may appear qualitatively similar to the normal (with the exception of vascularity and fibrous tissue of the pars anterior) but vary considerably in quantity both relative and absolute.

Tables 4 and 5 illustrate by a comparison of observed averages the absolute volumes of the normal and abnormal gland. The total weight of the abnormal hypophysis for the fifth to the sixth fetal months exceeds the weight of the normal gland by 12.2 mg. When the average weight is corrected for blood in the pars anterior it checks with the corrected weight value for the normal. The average weight for the sixth to the seventh fetal months is greater than the normal by 16.1 mg. When these averages are corrected for vascular-

ity, the anencephalic exceeds the normal weight by 0.65 mg. The absolute averages for the seventh to the eighth fetal months are

TABLE 5

A Comparison of the Average Total Weight of the Anencephalic Hypophysis with the Normal Fetal Hypophysis after each has been Corrected for Vascularity

Age in fetal months	Number of cases	Observed average weights of the anencephalic hypophysis *mg.*		Number of cases	Observed average weights of the normal hypophysis *mg.*		Differences between the average weights as corrected for vascularity of the anencephalic and normal fetal hypophyses *mg.*
Range		Uncorrected	Corrected		Uncorrected	Corrected	
5 to 6	6	42.3	26.35	8	30.10	26.35	± 0.00
6 to 7	6	58.5	36.10	16	42.4	35.45	+ 0.65
7 to 8	4	55.2	34.20	12	55.5	47.77	−13.57
8 to 9	..	...	....	13	81.2	67.89	....
9 to 10	..	...	....	12	95.6	79.93	....
10.16	1	113.2	71.80	1	112.6	98.12	−26.32

TABLE 6

Relative Volume of the Blood in the Pars anterior of the Normal Fetal Hypophysis

Necropsy number	Total body length *cm.*	Relative volume of blood *per cent*	Relative volume of remainder of pars anterior *per cent*
24–165	28.0	17.97	82.03
24–25	31.0	12.15	87.85
24–24	38.0	9.86	90.14
23–849	39.0	26.87	73.12
22–323	45.0	10.74	89.26
24–795	45.5	18.82	81.18
24–72	46.0	16.71	83.29
23–224	49.0	12.12	87.88
1746	51.5	21.36	78.64
23–408	55.0	16.41	83.59
23–535	60.0	17.13	82.87
Mean		16.40	83.60

about the same. The corrected values show the normal to be more than the anencephalic hypophysis in volume by 13.57 mg. Since there are no specimens between the age limits of the eighth and the

tenth fetal months, a comparison is not justified. The weight of the postmature specimen is about the same when compared with a normal of the same age. After correction for vascularity the normal exceeds the total weight of the gland in anencephaly by 26.3 mg.

It is obvious that if the amount of blood in the pars anterior be considered, the gland of the abnormal fetus usually is less in weight than the normal from a fetus of corresponding age. The same conclusion holds for the weights of the anterior lobes of normal and abnormal fetuses. The average relative amount of blood present in the normal fetal hypophysis is 16.4 per cent for eleven determinations. This figure is nearly two and one-half times less than the average for the pars anterior of the anencephalic gland.

Summary

The results obtained by this study may be summarized as follows:

1. An hypophysis is present in anencephalic fetuses.
2. It is extremely variable in weight. If the weight is corrected for vascularity it is usually less than the weight of the normal fetal hypophysis which has also been corrected.
3. The pars nervosa is lacking in the majority of cases. When present its relative and absolute volumes are considerably less than those of the normal.
4. The pars intermedia is variable in both occurrence and volume. It may be present in a gland in which there is no pars nervosa in evidence.
5. The pars anterior comprises most of the gland volume and apparently the total gland volume in some instances.
6. The average relative volume of blood present in the anterior lobe is about 39 per cent of the volume of that lobe.

The writer wishes to acknowledge his indebtedness to Professors R. E. Scammon and A. T. Rasmussen for their many valuable suggestions during the course of this study.

REFERENCES

1. Ballantyne, A. Manual of ante-natal pathology. Edinburgh, 1902–04.
2. Schwalbe, E. Die Morphologie der Missbildungen des Menschen und der Tiere. Jena, 1909, iii, 108.
3. Haberfeld, W. Zur Pathologie des Canalis craniopharyngeus. *Frankfurt. Ztschr. f. Path.*, 1910, iv, 96.

4. Brown, F. J. The anencephalic syndrome in its relation to apituitarism. *Edinburgh Med. J.*, 1920, N. S. xxv, 296.

5. Mauksch, H. Das Verhalten der Hypophyse und des Canalis craniopharyngeus in neun Fällen von Kranioschisis untersucht. *Anat. Anz.*, 1921, liv, 248.

6. Kohn, A. Anencephalie und Nebenniere. *Arch. f. mikro. Anat. u. Entwcklngsmechn.*, 1924, cii, 113.

7. Wrete, M. Ein Fall von Encephalo-Myeloschisis totalis bei einem menschlichem Embryo in der 7. Woche der Schwangerschaft. *Ztschr. f. mikro. Anat. Forsch.*, 1924, i, 563.

8. Nañagas, J. C. A comparison of the growth of the body dimensions of anencephalic human fetuses with normal fetal growth as determined by graphic analysis and empirical formulae. *Am. J. Anat.*, 1925–26, xxxv, 455.

9. Scammon, R. E., and Calkins, L. A. Simple empirical formulae for expressing the lineal growth of the human fetus. *Proc. Soc. Exper. Biol. and Med.*, 1922–23, xx, 353.

10. Rasmussen, A. T., and Herrick, R. A method for the volumetric study of the human hypophysis cerebri with illustrative results. *Proc. Soc. Exper. Biol. and Med.*, 1921–22, xix, 416.

11. Covell, W. P. Quantitative studies on the human prenatal hypophysis and hypophyseal fossa. (Abstract.) *Anat. Rec.*, 1926, xxxii, 230.

SYNGENESIOTRANSPLANTATION IN THE GUINEA-PIG *

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In a former paper we compared syngenesiotransplantation with homoiotransplantation and furthermore we compared with each the effect of exchange of pieces of tissues or organs between brothers, or of the transplantation of pieces from parent to children or from children to parents.[1] In general, syngenesiotransplantations behaved in a way intermediate between auto- and homoiotransplantation; however, there were considerable differences between the syngenesiotransplantations in individual cases. In addition there seemed to be differences between the following types of transplantation: I, brother to brother; II, children to parents; and III, parents to children. Type I gave the best results, type II gave the worst results and the results in type III were intermediate between those in type I and type II. However, the number of transplantations from parents to children and from children to parents was limited and it was unsafe to draw conclusions from a small number of experiments. We therefore decided to add to these various series of transplantations other experiments in order to obtain more definite data concerning syngenesiotransplantation. We not only increased the number of experiments, but we also extended them by including transplantation of tissues of grandchildren to grandparents and *vice versa*, and by increasing the number of organs used for transplantation.

In order to be able to summarize our results and to compare them with the results of homoiotransplantation, it will be necessary to use a method, similar to the one applied by us previously on various occasions, of grading the conditions found in the transplant. It will be of value to know the grades in the individual transplantations as well as the average grade of the various kinds of transplantations. The former indicate the degree of variations in syngenesiotransplantations as compared with those found in other kinds of transplantations. However, there is one variable factor which complicates the

* Received for publication October 7, 1926.

grading, namely, the length of time during which the tissues were allowed to remain in the strange host. The same state of preservation of the transplant and the same intensity of reaction observed after twenty days and after forty days would indicate different results; provided that no adaptation takes place between host and transplant, the reaction against the transplant should be more intense and the tissue more injured at forty than at twenty days. Furthermore, the reactions are more pronounced against certain tissues than against others. An absolutely exact grading would require that the effect of the tissue factor should be taken into account in a quantitative manner and that the same organs should be compared. This it has not been possible to do in our experiments up to the present, so far as the tissue factor is concerned. The grades therefore can only have a significance which is restricted by these limitations. But if we compare the findings in various series at the same period, results should be obtained which are approximately accurate. The grades which we use are as follows: *Grade 1*. The thyroid gland has completely or almost completely been destroyed. Fibrous tissue with some lymphocytes is left. The fat tissue around the cartilage transplant has been almost entirely replaced by fibrous tissue. There is a distinct lymphocytic infiltration around the cartilage. *Grade 2*. By far the greater part of the thyroid has been destroyed; some isolated acini and also a few small parcels of adjoining acini have been preserved. Most of the transplant consists of fibrous tissue and lymphocytes. The fat tissue around the cartilage is to a considerable extent invaded and replaced by fibrous tissue; there is a definite lymphocytic infiltration around the cartilage. *Grade 3*. The center of the transplanted thyroid consists of a great amount of dense fibrous tissue infiltrated with lymphocytes. It is in places surrounded by a thin ring of acini in which there is some lymphocytic infiltration. There is a certain amount of connective tissue increase between a number of the acini, which may exert pressure on some of them. In other parts of the periphery, the acini have been destroyed. In the case of the cartilage and fat tissue transplant, a fine distinction between grades 3 and 4 is not readily made. While in grade 2 the cartilage shows the characteristics just stated and in grade 5 the fat tissue is on the whole well preserved and almost or entirely free from fibrous tissue invasion (though it contains some collections of lymphocytes which may also be found in places around

the cartilage), in grades 3 and 4 the finding is on the whole intermediate between these two conditions. In grade 4, the center of the thyroid is fibrous and shows lymphocytic infiltration. There is a more or less well preserved ring of acini surrounding the central connective tissue; a distinct lymphocytic infiltration is found between or in the acini in places. There is also usually here and there some increase of fibrous tissue. Grade 5 corresponds to the typical picture in syngenesiotransplantation. The center of the transplant is not fibrous, but on the whole it shows loose, well vascularized connective tissue. It is surrounded by a well preserved ring of acini which are in good condition; they lie close together and contain for the most part colloid, in contrast to grades 2, 3 and 4, where a larger or smaller number of acini have lost the colloid. There may be occasionally a very slight increase in the amount of fibrous tissue in the center and in the periphery. This condition corresponds to the grade in a typical case of favorable syngenesiotransplantation. Grade 6 corresponds to the findings in autotransplantation. The center of the thyroid is vascular and relatively small; the ring of acini is well developed. Lymphocytes are absent or almost absent. There is no proliferation of connective tissue. The fat tissue around the transplant is well preserved. Increase in connective tissue and collections of lymphocytes are lacking in the fat tissue and around the cartilage. In some cases intermediate conditions are seen which are expressed through intermediate grades. There is a distinct correspondence between the conditions found in various kinds of transplants taken from the same donor and transplanted into the same host. But, as we stated on previous occasions, different tissues and organs differ in the intensity with which they call forth the lymphocytic and connective tissue reactions. Those tissues and organs which elicit these reactions most readily, are the finest indicators of genetic differences between the differentials of host and donor, while tissues and organs like cartilage and ovary, which are less readily attacked and destroyed, are less serviceable as indicators. The lack of responsiveness on the part of certain tissues may in certain cases bring about an apparent slight discrepancy between the reactions of various simultaneous transplants, but on the whole there is, as stated, a remarkable correspondence between them.

Series I. Transplantation from brother to brother. Thirty transplantations were made. The pieces were removed for examination at

various periods from fifteen to forty days after operation. Of these thirty cases, six behaved like autotransplants or approached autotransplants; twelve showed the character of syngenesiotransplants and twelve cases were like homoiotransplants or approached homoiotransplants. The individual grades were as follows: *15 days:* 3 (?); *17 days:* 5, 6, 3.25, 3 (?); *18 days:* 5.5, 6, 3 (?), 4; *20 days:* 2, 4, 6; *25 days:* 1; *26 days:* 5; *28 days:* 4, 2; *29 days:* 5; *30 days:* 4, 3, 2, 5.5; *33 days:* 5; *35 days:* 1, 4.75, 2, 2, 3.25, 1; *40 days:* 3.75, 6. The average of these thirty pieces is 3.7. As illustrations, the following abstracts of records may be cited.

(*a*) *40 days. Thyroid.* Many acini with colloid preserved, but separated by lymphocytes and fibrillar connective tissue. Center of transplant filled with lymphocytes. Over a wide area acini have been destroyed. *Cartilage.* Well preserved and surrounded partly by areolar tissue and partly by fibrous tissue. Much lymphocytic infiltration in areolar as well as in fibrous tissue. Grade 3.75.

(*b*) *40 days. Thyroid.* Excellent preservation; acini with low to medium-sized epithelium, close together with solid, retracted colloid. In center of transplant some areolar and connective tissue and a small collection of lymphocytes. *Cartilage.* Well preserved and surrounded by areolar tissue and some fine fibrillar connective tissue; no lymphocytes. Grade 6.

(*c*) *33 days.* Transplants taken out soon after death of animal. *Thyroid.* Almost like autotransplant, but increased lymphocytic infiltration at points in periphery and in places in center, from here penetrating between some acini and surrounding them. Lymphocytic masses enter also into epithelium of some acini. Some lymph vessels filled with lymphocytes. Some acini in process of destruction. The center of transplant is composed of areolar and some dense fibrous tissue; it is surrounded by a ring of thyroid tissue. With the lymphocytes, connective tissue cells penetrate between the acini. In this case the transplant had at first the character of an autotransplant; secondarily some connective tissue cells with considerable collections of lymphocytes penetrated beneath and between the acini and separated them from each other. *Cartilage.* Much cellular cartilage. The transplant is surrounded by areolar tissue, the septa of which are somewhat thickened. There is a slight new formation of connective tissue, with a few lymphocytes. Grade 5.

(*d*) *30 days. Thyroid.* Ring of well preserved acini. In center large vessels surrounded by edematous connective tissue. Medium-sized acinus cells. Acini close together and with colloid; in other places widely separated by lymphocytes. Frequent mitoses in acinus and connective tissue cells. In places also compression and destruction of acini and acini without colloid, though in certain areas many acini may be left. Some acini filled with cells. Lymph vessels in center filled with lymphocytes. Grade 4.

(*e*) *26 days. Thyroid and parathyroid.* Very well preserved. Thyroid consists of large masses of acini which are close together and have solid colloid. Relatively little connective tissue in center of transplant, but much lymphocytic infiltration here and at one pole of the periphery. At numerous points lymphocytes begin to penetrate between acini. In center also lymph vessels filled with lymphocytes. Around thyroid and parathyroid some groups of lymphocytes, which in various places just begin to infiltrate thyroid. *Ovary.* Good primordial, small and medium-sized follicles with follicular cavities. Good ova in center. Mitoses in granulosa of follicles. No follicles with degeneration of granulosa; therefore no dividing egg. In some small follicles without cavity eggs are being destroyed. In center large areas of necrotic tissue organized by loose connective tissue. Atretic yellow bodies. Cavities of atretic follicles. Wide medullary canals; in places some marked lymphocytic infiltration in periphery. Lymphocytes are also in cortex and encroach upon some primordial follicles, but the latter are, on the whole, well preserved. No germ epithelium left. In periphery, part of corpus luteum is preserved, but it is somewhat vacuolar and moderately infiltrated with lymphocytes. Some polymorphonuclear leucocytes in cortex of ovary among the lymphocytes. On the whole, considerable lymphocytic infiltration; the lymphocytes are found in groups here and there and also almost in a continuous ring. The lymphocytes cannot penetrate to periphery of central fibrous areas. Grade 5.

(*f*) *20 days. Thyroid.* In the center which is small are large vessels and loose edematous connective tissue. Excellent ring of acini with much colloid. Solid colloid retracted. Acini close together, well vascularized. Some acinus cells take up blood pigment. A small number of lymphocytes around central vessels. No lymphocytes migrate through thyroid. Grade 6.

These six cases differ from those which are characteristic of

homoiotransplants. The centers show the structure of autotransplants, indicating that in the first period following transplantation the host tissue behaved toward the transplant as if it had been autotransplanted tissue; but subsequently the lymphocytes are attracted by the transplant, and they invade and partly destroy it. At the same time a moderate new formation of connective tissue takes place. The lymphocytes migrate into the living, actively metabolizing, and not into dying thyroid. In other cases, the syngenesio-toxins are produced in so great a dilution, that the transplant acts like an autotransplant as late as forty days after transplantation. Still other transplants resemble homoiotransplants, behaving similarly to the transplants described in our preceding papers on homoiotransplantation. We see that the condition of the cartilage transplant is parallel to that of the thyroid transplant, although the reactions against the cartilage and fat transplant are less pronounced than those against the thyroid. The transplanted ovary is well preserved; the follicles develop to a certain size and contain normal ova. Lymphocytes invade also the ovary, but the infiltration does not become very intense. In two cases host and donor were the offspring of parents one of which was smooth haired, while the other one was curly haired. The grades in these two cases were 4 and 2.

Series II. Transplantations from children to parents. In some cases only thyroid, in other cases both thyroid and cartilage were used for transplantation. Altogether 104 transplantations were made in this series. Between seven and sixteen days (inclusive) after transplantation, thirteen cases. *11 days:* approaching homoio-character. *12 days:* (*a*) decided syngenesio-homoio-character; (*b*) syngenesio-character. *14 days:* (*a*) approaching homoio-character; (*b*) through infection the greater part of thyroid destroyed. *15 days:* (*a*) homoio-; (*b*) syngenesio-; (*c*) homoio-; (*d*) homoio-; (*e*) homoio-character. *16 days:* homoio-character, with intense lymphocytic infiltration. *17 days:* 8 cases; *18 days:* 9 cases; *19 days:* 19 cases; *20 days:* 22 cases; 21 *days:* 6 cases; *22 days:* 4 cases; *24 days:* 2 cases; *25 days:* 8 cases; *26 days:* 2 cases; *27 days:* 2 cases; *28 days:* 3 cases; *29 days:* 2 cases; *31 days:* 4 cases.

If we omit the case taken out and examined seven days after transplantation and one other instance in which some special conditions were present, we may classify the remaining cases as follows: Approaching auto-character: 6 cases. Good syngenesio-character: 6

cases. Syngenesio-character: 10 cases. Decided syngenesio-character: 6 cases. Syngenesio-homoio-character: 24 cases. Homoio- or approaching homoio-character: 50 cases; some of these were doubtful, no thyroid being found. Altogether favorable transplants (syngenesio- to approaching auto-character): 22 cases. Unfavorable cases (homoio- to bad syngenesio-character): 80 cases.

If we grade the cases from seventeen days to thirty-one days (inclusive) after transplantation, we find the average grade of the ninety-one cases is 2.91, which is decidedly below that obtained in the brother to brother transplantations. The individual grades vary between 1 and 6.

Grades at *17 days:* 3, 3.5, 3.5, 2, 5, 5, 3.5. Grades at *18 days:* 2.5, 2.25, 6, 3, 4.5, 4.5, 4.5, 1, 4.5. Grades at *19 days:* 5.5, 5.75, 4, 4, 3.5, 2, 1 (?), 3, 3.5, 1 (?), 1.5, 3.5, 4, 3.5, 4.5, 1 (?), 1 (?), 3.25, 3.25. Grades at *20 days:* 3, 3, 1, 1.5, 2, 4, 3, 5.5, 6, 1.5, 4.5, 3, 4, 4, 3.5, 1, 4, 1 (?), 1 (?), 4.5, 3.5. Grades at *21 days:* 1, 2, 3.75, 3, 1, 3. Grades at *22 days:* 5.75, 3, 2.5, 4. Grades at *24 days:* *2, *2. Grades at *25 days:* *1, *2, *1, *2, *2 (?), 1 (?), 6, 3. Grades at *26 days:* *1, 2. Grades at *27 days:* *1, *1. Grades at *28 days:* *3 (?), 5, 1. Grades at *29 days:* 2.5, 2.5. Grades at *31 days:* *3, 3 (?), 1.5, 1.5.

In those cases in which the grade has an asterisk, the parents showed more marked differences than in the rest of the cases, one parent having smooth and the other rough hair. Twelve such transplantations were made, the average grade being 1.7, which is considerably below the average grade in this series. This indicates that the reaction in the parents against the tissues of a child carrying these strange individuality differentials is greater than that against the tissues of a child, both parents of which had smooth hair. On the other hand, in a number of instances in which very good results varying between auto- and syngenesio-character were obtained, there is some indication that the parents were related to each other. Thus in four out of six cases in which the result approached an auto-condition, the parents were in all probability related to each other. In the two remaining cases, this was not certain. Among the sixteen cases in which a syngenesio-reaction was obtained, in seven cases there was a definite indication or a suggestion that the parents were related to each other. Thus the type of reaction obtained in transplantation from children to parents depends upon the relationships of the individuality differentials of host and donor.

Twenty-nine experiments were arranged in the following manner. In certain cases a thyroid lobe, with or without a piece of xiphoid cartilage, of a child was transplanted to the father and to the mother; in other cases a thyroid lobe and piece of cartilage were transplanted from a child to one side of mother or father and from a second child to the other side of the same parent. The pieces were taken out at the same time for examination. Our aim was to determine how often the tissues of both parents would behave in the same way toward the tissues of the same child and how often they would behave differently; also how often one parent would behave in a like manner or differently to the tissues of two children.

The results were as follows: Two of these twenty-nine cases have to be discarded because in one host the transplant was not found. In seventeen cases the transplants of a pair behave in the same way or in almost the same way; in all except two of these pairs a homoio-reaction or a condition approaching it was obtained. In one case both members of a pair behave like autotransplants and in another case like decided syngenesiotransplants. In four pairs the two members behaved differently. In two cases one member of each pair showed an auto-reaction and the other a reaction standing between syngenesio- and homoio-reaction; in the other two cases a homoio-reaction was combined with a good syngenesio-reaction. In six cases the pairs differed, but the difference was less marked than in the former two cases. Considering the preponderance of homoio-reactions in this series we must expect to find in many cases homoio-reaction in both transplants. We may then conclude that in a number of cases the two partners of a pair behave differently, that the individuality differentials of brothers differ or that the individuality differential of a child has a greater similarity to the individuality differential of one parent than to that of the other parent. While in many cases in this series the result corresponds to or approaches a homoio-reaction, still it differs from that in the series of homoio-transplantations, since there are in the latter exclusively homoio-reactions or reactions approaching this condition and in the former a considerable number of auto- and syngenesiotransplantations are added to the homoio-reactions. The average grade is therefore higher in the present series. We may cite some examples of transplantations from children to parents. (*a*) *18 days*. From 12–13 days old child to mother. *Thyroid*. Much dense fibrous tissue, but no

wide vessels in center. Very incomplete ring of acini; almost two-thirds of thyroid is destroyed. Acini separated by much diffuse lymphocytic infiltration; mitoses in acini which are attacked by lymphocytes. In large majority of acini the colloid destroyed by phagocytes, but some plates of colloid left. Grade 2.75.

(*b*) *18 days.* From child to father. *Thyroid.* In center of transplant some dense fibrous tissue, but in the periphery of center edematous connective tissue and large vessels penetrating through thyroid ring into center. Good ring of acini containing colloid and lying in close approximation to each other. The *parathyroid* transplant also well preserved; some loose connective tissue in center. Lymphocytic infiltration lacking. Grade 6.

(*c*) *19 days.* From child to father. *Thyroid.* Large lymph and blood capillaries in periphery of center. Rather intense lymphocytic infiltration in center, from here penetrating into thyroid ring. An area of well preserved acini, close together and containing well formed colloid; but part of thyroid destroyed. Grade 3.75.

(*d*) *25 days.* From child to father. Mother was Abyssinian, father smooth haired. Child has some characters from father and some from mother. *Thyroid.* Only fibrous tissue, with blood pigment and lymphocytic infiltration, found; no thyroid tissue left. *Cartilage.* Fibrous tissue and very marked lymphocytic infiltration surrounding cartilage. End of cartilage consists of cartilage cells with very little hyaline substance separating them; here lymphocytes deeply invade cartilage and injure it.

Series III. Transplantations from parents to children. In this series thirty-three transplantations were made; two transplants were examined as early as seven and almost nine days after transplantation; the other pieces were taken out after periods varying between ten and thirty-two days. The results in all except the first two cases were as follows: auto-reaction in 4 cases (10, 14, 17 and 20 days after transplantation); syngenesio-reaction in 2 cases (18 and 20 days); marked syngenesio-reaction in 2 cases (20 and 30 days); homoio-reactions or reactions approaching these in 23 cases (14 to 32 days). In one-sixth of the cases auto- or syngenesio-reactions were obtained. The grades are as follows: *10 days:* 6; *14 days:* 4, 2.5 (?), 6; *17 days:* 6; *18 days:* 1 (?), 2.5, 4.5; *20 days:* 5.75, 1.5, 3.5, 4; *21 days:* *1.5, *2; *25 days:* *1, *1, *1.5, *1, *2, *2, *1 (?); *29 days:* 1, 1; *30 days:* *3.25, *1.5, *3, *2.75, 1.5, 2.5; *32 days:* 3.25, 1.5. The

average for the series is 2.6. In thirteen cases, marked with an asterisk, where one parent was smooth and the other rough haired, the average grade is 1.8. The reaction is therefore stronger in these cases and the injury of the transplant greater. This is the same result which we obtained in the transplantation from children to parents. In nine cases in this series, pairs were used for transplantation similar to those we discussed in the transplantation from children to parents. In some cases the thyroid lobe of one side and piece of cartilage were transplanted to one child and the other lobe and piece of cartilage to a second child; or in other cases a thyroid lobe and a piece of cartilage from one parent were transplanted into the right side and from the other parent into the left side of the same child. In all cases the paired transplants behaved in the same or in a similar way; in seven instances a homoio-reaction was obtained in both members of the pair. This was to have been expected considering the large number of homoio-reactions or reactions approaching them in this series.

The following examples may be cited: (*a*) *20 days. Thyroid.* Incomplete ring of acini. Much lymphocytic infiltration in center and between acini; also increased fibrillar connective tissue between acini; in some cases lymphocytes enter the colloid in the center of acini and destroy it. In places acini are still close together. *Cartilage.* The transplant surrounded by loose areolar connective and fat tissue. There is some lymphocytic infiltration in the fat tissue around cartilage and it extends farther out into fat tissue. At both ends of the cartilage there is some regenerated cartilage. Grade 4.

(*b*) *25 days.* Father smooth, agoute; mother, rough haired, red; child, rough haired, agoute and red. From father to child. *Thyroid.* Instead of transplant only hyaline fibrous material with some lymphocytes is found; no thyroid left. *Cartilage.* Partly alive and partly necrotic; especially where it is thick we find in the center some pyknosis and loose cells. The piece is surrounded by fibrous tissue which includes some remaining fat cells. Very moderate lymphocytic infiltration around cartilage. Grade 1.

Series IV. Transplantation from grandchildren to grandparents. This series as well as the one following is smaller than the preceding series of syngenesiotransplantations. Thirteen experiments were carried out; the examination took place from the twentieth to the twenty-seventh day after operation. In two of these cases the result

was an auto-reaction, while in the eleven other cases the results approached or were definite homoio-reactions. In some instances only cartilage was transplanted and then the cases were difficult to grade since, as we have stated on previous occasions, cartilage is a less delicate indicator than thyroid of the reaction on the part of the host toward the transplant. With this reservation we give the following list of grades which we obtained in this series: 6, 5.75, 3, 2.75, 2.25, 2 in six cases, 1.5, 1. While these grades are relatively low, still they are better than those obtained in homoiotransplantations.

In four cases pairs were examined in the same way as in the preceding series; in three of these pairs the results were the same, the grades corresponding to or approaching a homoio-reaction. In one case, a splitting of characters took place. The grandfather was an Angora guinea-pig, and the grandmother was smooth; the grandchild was an Angora and resembled therefore the grandfather. In this case the tissue that had been transplanted into the grandfather resembled an autotransplant, while the tissue that had been transplanted into the grandmother resembled a homoiotransplant. The individuality differentials in this instance corresponded to the similarities of appearance and race characteristics. If we exchange tissues between hybrids and ancestors belonging to different races, an auto-reaction should be obtained in a certain proportion of cases.

Series V. Transplantation from grandparents to grandchildren. In this series seventeen transplantations were made; the pieces were taken out for examination from twenty to twenty-seven days after operation. The results were as follows: An auto-reaction was obtained in two cases, a syngenesio-reaction in five cases and a reaction approaching or equal to a homoio-reaction in ten cases. Grades: *20 days:* 2, 4.5, 5.5, 2, and in one case, probably of homoio-character, it was impossible to determine the grade; *22 days:* 1.5, 1, 1, 4.5, 4.5; *24 days:* 4, 2, 2.5; *25 days:* 1, 1.5; *27 days:* 4.75, 6. The average of the grades is 3. The average results are better than in homoiotransplantations owing to the number of auto- and syngenesio-reactions obtained. In seven instances, pairs were compared. The results in the members of the same group were usually similar. In four pairs all the pieces showed or approached homoio-character. In one pair both transplants were very well preserved and called forth only a very slight reaction. In one pair the transplants from one member showed an auto-, and from the other member, a syngenesio-reaction;

in this case some difference in the behavior of the two partners was therefore observed.

Conclusions

The reactions against syngenesiotransplantations in the guinea-pig are intermediate in intensity between auto- and homoio-reactions. However, this statement is correct only so far as the averages are concerned; the individual reactions may either approach or reach auto-reactions, on the one hand, and on the other, homoio-reactions. There are, however, typical intermediate reactions which we may designate as syngenesio-reactions in the strict sense. They occur when the individuality differentials between host and transplant are not identical but are so much related to each other that toxins develop late and not at a very early stage after transplantation. It seems that some time must elapse before a relatively slight incompatibility between the differentials permits a concentration of injurious substances sufficient to cause the disturbances. Thus in the first period after transplantation (in the case of thyroid) between the sixth and twelfth day, when the general structure of the transplant is laid down, the absence of a sufficient amount of individuality toxins allows the development of auto-structure; the connective tissue is, to a large extent, loose in the center and small in amount; the vascularization is good and the thyroid ring develops well; connective tissue is not increased between the acini and lymphocytes are absent. Then gradually when the concentration of toxins becomes sufficiently strong, lymphocytes appear, fill the lymph vessels and invade the transplant. These cells collect especially in large numbers at the inner edge of the thyroid ring; from here they penetrate between and also into the acini, which are still perfectly well preserved, and help to destroy them. There may now probably develop secondarily some connective tissue between the acini. Between this type of reaction which we observed in a number of cases, and the typical homoio-reaction on the one hand, and the auto-reaction on the other hand, all kinds of intermediate types exist. When tissues other than thyroid are transplanted, conditions are similar; but the reactions of the host against cartilage are not so sharply demarcated from each other as they are against thyroid. In the case of the cartilage transplant the amount of fibrous tissue development in the fat and areolar tissue, and the degree of lympho-

cytic reaction in the fat tissue and around the cartilage are indicators of the intensity of the reaction. There is, on the whole, a good agreement between the various reactions if different kinds of tissues are transplanted simultaneously from the one donor into the same host. There are then two ways in which the intermediate average between homoio- and auto-reaction is brought about in the case of syngenesiotransplantations, namely, (1) in the first place, reactions approaching both auto- and homoio-character occur in different cases and as far as the average result is concerned compensate each other, and (2) in a number of host-transplant combinations actual intermediate reactions take place. The averages in the different types of syngenesio-reactions were as follows:

1. Brother to brother transplantation, 3.7.
2. Children to parents transplantation, 2.91.
3. Parents to children transplantation, 2.60.
4. Grandparents to grandchildren transplantation, 3.
5. Grandchildren to grandparents transplantation, 2.6.

The brother to brother transplantations give the best results. The transplantations of parent to children, grandparents to grandchildren and *vice versa* give results not so good as those from brother to brother; but all are intermediate in character, although the averages of the transplantation taking place between members of different generations are distinctly nearer the homoio-type than the auto-type. If we assume that the individuality differentials are composed of a considerable number of factors, perhaps a number corresponding to the number of the chromosomes except the sex chromosomes, and that the presence in the transplant of factors strange to the host causes indirectly the reaction against the transplant, then we must expect that brother to brother transplants should on the average call forth the least intense reactions. As to the transplantations from parent (grandparents) to children (grandchildren) they should both lead to more marked reactions. In one case the total average was somewhat better in the transplantations from children to parents than in the reverse transplantations from parents to children. On the other hand, the total average was slightly better in the series in which pieces were transplanted from grandparents to grandchildren than in the reciprocal series. These differences are probably to a large extent due to the composition of the families used for transplantations. Exchange of tissues between

different generations should give poorer results if the individuals used are not at all related or if they belong to different races, than those obtained in families where some inbreeding has taken place. This probably explains the fact that we obtained the lowest average grade in transplantations which were made from parents to children; in this series there were a number of families where one of the parents had smooth, the other rough hair.

These results agree with our former investigations in that they confirm the intermediate position of the average grade for syngenesiotransplants as well as the intermediate character of the syngenesio-reaction proper. They confirm also our conception of the mode of syngenesio-reaction and the part connective tissue, blood vessels and lymphocytes play in this reaction. But at the same time we carried our analysis further by experimenting on a much larger scale than we did previously; we showed the relative frequency of the various types of reactions in each kind of syngenesiotransplantation. Furthermore, we found reasons for assuming that the character of the parents (the degree of their relationship or race differences existing between them) influences the results of syngenesiotransplantation. While we thus found, as in our previous investigations, that brother to brother transplantations have the highest average grade, we did not obtain very far-going differences between parents to children and children to parents transplantations. Such differences as we observed were probably, at least in a measure, due to genetic relationships or differences between the parents. Thus we supplement and correct some of our previous figures which were in part based on a limited number of experiments.

If we compare the individuality differentials of both parents with the individuality differential of their child, or if we compare the individuality differentials of two brothers with that of one of their parents, we find that the members of a pair may behave differently; but in the majority of cases they behave similarly. This might be expected if we consider the fact that in so many cases homoioreactions obtain; according to the law of probabilities a considerable number of individuality differentials of pairs should be similar under these circumstances.

Summary

1. Syngenesio-reactions in the guinea-pig have a position intermediate in character between homoio- and auto-reactions. This intermediate character is due to the variations in individual reactions which approach auto-reactions, on the one hand, and homoio-reactions, on the other hand, as well as to the existence of a real intermediate syngenesio-reaction.

2. The typical intermediate syngenesio-reaction resembles the auto-reaction as far as the behavior of connective tissue and vessels is concerned. It differs from the auto-reaction in as much as subsequently lymphocytic masses invade the transplant.

3. Brother to brother transplantations resemble autotransplantations to a higher degree than parent to children or the reciprocal transplantations. The grade of transplantations from parent to children does not seem to be significantly higher than the grade for the reciprocal transplantations.

4. Transplantations between grandparents and grandchildren have average grades similar to those of transplantations between parents and children.

5. Relationship or dissimilarity (race differences) between the two parents seems to influence the intensity of reactions in the exchange of tissues between the various members of families.

REFERENCE

1. Loeb, Leo. *J. Med. Res.*, 1918, xxxix, 39.

SYNGENESIOTRANSPLANTATION IN THE RAT *

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In the preceding paper of this series we discussed the results obtained in syngenesiotransplantation in the guinea-pig. We have carried out corresponding experiments in the rat and we shall discuss these in the present paper, thus extending previous experiments in this species, in which multiple syngenesiotransplantations of various organs were made.

In order to summarize our findings we shall use the same set of grades which we defined in our preceding paper.

SERIES I. TRANSPLANTATION FROM BROTHER TO BROTHER

(1) *19 to 25 days after transplantation.* Thirteen experiments in which pieces often of several organs were transplanted into the same animal. In ten cases the reactions approached or were identical with auto-reactions; in the three remaining cases the reactions were not so good, but still were better than homoio-reactions. Grade 6 in 5 cases; grade 5.75 in 3 cases; grade 5.5 in 1 case; grade 5.25 in 1 case; grade 4.5, grade 3.25 and grade 3, each in 1 case. Average grade 5.29.

(2) *26 to 35 days (incl.) after transplantation.* Twenty-three experiments. In eleven cases the reactions approached or were identical with auto-reactions; in twelve cases the reactions ranged between those characteristic of syngenesio- and of homoiotransplantations. In various cases there was a very moderate or strong lymphocytic reaction present. Grade 6 in 3 cases; grades 5.75 and 5.50 in 4 cases; grades 5.25 and 5 in 6 cases; grade 4 in 3 cases; grades 3.75, 3.50 and 3 in 3 cases; grades 2.75, 2.50 and 2.25 in 4 cases. Average grade 4.49. In this period the intensity of the lymphocytic reaction increases somewhat and on the whole the more so, the later the period of examination. The lymphocytic infiltration can be present without a concomitant connective tissue reaction.

* Received for publication October 7, 1926.

(3) *40 to 60 days after transplantation.* Sixteen experiments. In eight cases the reactions approached or were identical with an auto-reaction. Moderate lymphocytic reaction in two cases; much lymphocytic reaction in four cases; homoio-reaction in two cases. Grade 6 in 5 cases; grade 5.50 in 2 cases; grade 5 in 1 case; grade 4.5 in 2 cases; grades 3.75 and 3.25 in 3 cases; grade 2.75 in 1 case; grades 1.5 and 1 in 2 cases. Average grade 4.41.

If we compare the average reactions in different periods we find between 19 and 25 days, grade 5.29; 25 and 35 days, grade 4.49; and between 40 and 60 days, grade 4.41. The average grade of the fifty-two experiments (examination between 19 and 60 days) is 4.66.

In twenty-five additional experiments the pieces were taken out at earlier periods, namely between eight and seventeen days. It is difficult, especially between the eighth and fourteenth days to assign grades to the reactions; but if we attempt a grading at this early period, we may summarize the reactions as follows: grade 6 in 9 cases; grades 5.5 and 5 in 4 cases; grades 4.5 and 4.25 in 5 cases; grade 4 in 3 cases; grade 3 in 2 cases; grades 2.5 and 2 in 2 cases. The average grade of this group is 4.8.

In order to illustrate these figures we shall select especially cases in which a syngenesio-reaction was obtained, and in which, in addition to thyroid or cartilage, ovarian tissue or uterus and tube was transplanted.

(*a*) *19 days. Ovaries.* Large and small well preserved follicles with ova. There were well formed corpora lutea and among them new corpora lutea as well as a retrogressing corpus luteum; also primordial follicles with eggs were seen and adjoining them small collections of similar cells, but without eggs. Thick interstitial tissue in which large, yellow-stained cells were found. Tubes and uterine tissue, epithelial as well as muscle tissue, were well preserved. Grade 6.

(*b*) *19 days.* The structure of thyroid and cartilage corresponds to an auto-reaction, but in the thyroid transplant there are in a few places considerable collections of lymphocytes around vessels. In the cartilage transplant there are in the fat tissue a few small collections of lymphocytes, also some lymph vessels containing these cells. The number of invading lymphocytes is greater in the thyroid than in the cartilage transplant. The perichondrium produces a new plate of cartilage. Grade 5.5.

(*c*) *24 days.* Good thyroid transplant; acini with medium-sized epithelium and good solid retracted colloid. In the center some ducts and cell nests. Through solution of cell masses, cavities are produced in these cell nests. Around latter and around some central vessels there is a slight lymphocytic infiltration. Also at one pole of the thyroid there is a moderate mass of lymphocytes. Fibrous tissue surrounds large vessels. Well preserved parathyroid transplant. Grade 5.25.

(*d*) *25 days.* Thyroid transplant well preserved. In center some increase in fibrous tissue, but also a few fat cells. Transplanted artery preserved. In various places there are collections of lymphocytes in periphery and in center of transplant. Also individual acini are occasionally separated by lymphocytes. While collections of these cells are frequent, there are wide areas without lymphocytic infiltration but with well preserved acini containing solid colloid. In cartilage transplant much less lymphocytic infiltration than in thyroid. There is a large amount of areolar tissue around cartilage and some slight collections of lymphocytes are found in it; none of these cells in fibrous tissue around areolar tissue. Over wide areas the cartilage is necrotic. Separated from necrotic cartilage through necrotic fibrous tissue there are found sheets of new, living cartilage produced through perichondrial proliferation; this is observed in places on both sides of the cartilage, although not everywhere. Grade 4.5.

(*e*) *25 days.* In thyroid transplant acini are almost without colloid and without lumen. The acini are compressed and have high epithelium. Some loose, partly necrotic connective tissue in center; connective tissue grows also around acini. Large vessels grow into the center. Lymphocytes penetrate the acini and destroy them; but a great number of compressed acini are preserved. Of the uterus transplant only fat and fibrous tissue, with much lymphocytic infiltration, is found. In the transplant of the ovary there are well preserved primordial follicles and eggs and well preserved further developed follicles also with ova. In the granulosa many mitoses. Many atretic follicles and epithelial ducts. Some remnants of interstitial gland. Mitoses in germ epithelium cells; this layer of cells forms a cyst. Lymphocytic infiltration in ovarian stroma; some lymphocytes in granulosa epithelium and also in interstitial tissue; lymph vessels filled with these cells. There is a correspondence in behavior of thyroid and ovary transplants. Grade 3.

(*f*) *30 days.* Thyroid almost like autotransplant. A little fibrous tissue in center; well formed acini with colloid. Some loose connective tissue and blood pigment cells in center; large vessels here also; several small lymph vessels filled with lymphocytes. A few of the latter cells seen between, and in places entering, the acini. At one point outside of the thyroid rather dense collection of lymphocytes. Also some diffuse lymphocytic infiltration in fibrous tissue here and there in center. Mitoses in acinus cells. Well preserved parathyroid with a few lymphocytes in parathyroid tissue. The structure of the thyroid transplant is that of an autotransplant. Adjoining the cartilage transplant there is a large amount of fat tissue. Cartilage and perichondrium well preserved; where former has been injured, latter proliferates. Muscle tissue that attaches to cartilage, in good condition; occasionally in fat tissue, small collection of lymphocytes. Grade 5.25.

(*g*) *30 days.* Beginning pregnancy in host. Well preserved thyroid ring, colloid in acini. Some loose connective tissue and fairly marked lymphocytic infiltration in center; lymph vessels filled with lymphocytes. Also some diffuse and some more moderate lymphocytic infiltration in parathyroid. Very much lymphocytic infiltration in connective tissue and perichondrium around cartilage. Grade 4.

(*h*) *30 days.* Thyroid and parathyroid transplants well preserved; large acini with good colloid. Little connective tissue, some fat tissue and good vascularization in center. Lymph vessels in center filled with lymphocytes. Some localized lymphocytic collection in thyroid. Lymphocytes begin to infiltrate the transplant without any preceding connective tissue changes. Transplanted uterus and Fallopian tubes with well preserved epithelium, connective tissue and musculature. In the transplanted ovary, primordial follicles with eggs small and large and well developed follicles with ova. In some follicles degeneration of granulosa. Medullary ducts. Necrotic corpus luteum with yellow pigment. Interstitial gland. Around foreign body giant cells usually some lymphocytic collection. On the whole very slight lymphocytic infiltration in ovary. Grade 5.25.

(*j*) *30 days.* Thyroid transplant, with intense lymphocytic infiltration, appears almost like a lymph gland. Only here and there isolated small nests of acini are left. In the center there is somewhat loose connective tissue with moderate lymphocytic infiltration. A

number of acini without colloid and compressed; lymphocytes are around and in them. Also in the surrounding fat tissue and in the parathyroid there is some lymphocytic infiltration. In a short time probably the whole transplant would have been destroyed. Uterine epithelium, mucosa and muscularis preserved; there is some lymphocytic infiltration in the uterus proper, but the most marked infiltration is in the periuterine and in the fat tissue and here it is extreme; the lymph vessels are studded with lymphocytes. In the ovarian transplant a large germ epithelium cyst and interstitial tissue are visible. The greater part of lymphocytic infiltration is around the large lymph and blood vessels. Grade 3.

(*k*) *35 days.* Thyroid transplant with intense lymphocytic infiltration. Some irregular and scattered acini without colloid left in the center in the midst of lymphocytic masses. A few acini with good colloid are in periphery. In the center lymph vessels studded with lymphocytes. Blood vessels very conspicuous. Uterus transplant very large. A great deal of lymphocytic infiltration in uterine mucosa; surrounding lymph vessels filled with lymphocytes. Much lymphocytic infiltration between and around muscle tissue. Lymphocytes penetrate also into epithelium and partly injure it, but directly underneath epithelium there are only a moderate number of these cells found. Grade 2.5.

COMMENT. We see in these examples variations in the syngenesio-reactions from auto-reactions on the one hand to a condition approaching homoio-reactions on the other. In some cases the center of the piece behaves like that of an autotransplant, but subsequently a lymphocytic infiltration occurs, which in the course of time may become quite marked. In other instances there may be a little increase in fibrous tissue in the center and a more prominent lymphocytic reaction and finally, in still more pronounced cases, the increase in fibrous tissue may extend even around the acini; in the latter event the transplant usually approaches gradual destruction. There is again a parallelism in the behavior of cartilage and thyroid; but as in the guinea-pig the lymphocytic reaction around the cartilage is less marked than around the thyroid. The parathyroid also shows corresponding gradations of lymphocytic infiltration, although in this organ the infiltration is frequently not so marked as in the case of the thyroid. There is on the whole a parallelism in reactions against thyroid and uterus; but uterine tissue may occasionally be

more resistant to the action of syngenesio-toxins than is thyroid. Epithelium, mucosa and muscle layer may be preserved but later a lymphocytic infiltration, varying in strength, sets in. It may affect in some cases the tissue surrounding muscle and epithelium more than it affects these latter structures; but they also are invaded by lymphocytes. The ovarian tissue shows corresponding gradations in reactions. If there is a severe reaction against the thyroid, only certain of the more resistant parts of the ovarian tissue are preserved, such as the layer of germ epithelium (which may form a cyst), interstitial tissue and the medullary ducts. If the thyroid shows a typical syngenesio-reaction, all the ovarian structures, including primordial follicles and various kinds of Graafian follicles with ova are well preserved; atretic follicles also are found. Mitotic proliferation occurs in follicles. Corpora lutea, some of which are newly formed, are preserved. But in cases of syngenesio-reaction lymphocytes begin to infiltrate somewhat even the well preserved ovarian transplant. First they collect around vessels and gradually they may invade even the follicles. A parallelism is thus seen to exist in the intensity of reaction against thyroid and that against ovary, although on the whole the ovary resists better the consequences of syngenesio-toxin formation than the thyroid tissue.

In specimen (*f*) examined thirty days after transplantation, with a grade of 5.25, in which there was therefore a good syngenesio-reaction, striated muscle tissue was found well developed. This was also observed in other cases. Striated muscle tissue may regenerate after transplantation, at least if the toxic action is not pronounced. The conditions under which this regeneration occurs need further investigation.

Series II. Transplantation from Children to Parents

(1) *18 to 25 days after transplantation.* Twenty-six experiments. In nine of these cases an auto-reaction was reached or approached; in six cases there was a syngenesio-reaction with slight, in two cases with moderate, and in two cases with marked lymphocytic infiltration; in seven cases there was a homoio-reaction. If we include a few experiments in which the examinations occurred twelve to eighteen days after operation the individual grades are as follows: grade 6 in 4 cases; grades 5.75 to 5 in 13 cases; grades 4.75 to 4 in 4 cases;

grades 3.75 to 2.75 in 4 cases; grade 2.50 to 2 in 4 cases; grade 1 in 1 case. The average grade in these 30 cases is 4.4.

(2) *26 to 50 days after transplantation.* Twelve experiments. In five of these cases an auto-reaction was reached or approached; in one case there was a syngenesio-reaction with slight, in two cases with moderate, and in two cases with marked lymphocytic infiltration; in two cases there was a homoio-reaction. The grades are as follows: grade 6 in 4 cases; grades 5.7 to 5 in 2 cases; grades 4.75 to 4 in 4 cases; grades 2.75 to 2 in 2 cases. The average grade is 4.7. The average grade of the whole group of syngenesiotransplantations (children to parents) is 4.5. This average is not quite so good as the average of the brother to brother transplantations, but nearly approaches it; it is, on the other hand, much better than the average of the homoiotransplantations.

If we compare the reactions in the two types of syngenesiotransplantations between eighteen and twenty-five days, we find in the brother to brother transplantation in nineteen cases an auto-reaction or a slight lymphocytic infiltration, while in five cases the reaction was more severe. In the series of transplantation from children to parents, in nineteen instances we find an auto-reaction or slight lymphocytic infiltration, while a more severe reaction occurred in nineteen other cases; therefore in 50 per cent of the cases the reaction was more severe; while in the brother to brother transplantation the more severe reaction was obtained in only 21 per cent of cases. Between twelve and seventeen days, the relative proportions are similar, namely, auto-reaction or slight lymphocytic reaction in 73 per cent of the brother to brother and in 50 per cent of the children to parent transplantations. The more severe reactions were obtained in 23 per cent of the brother to brother and in 50 per cent in the children to parent series.

During the same period of time the reactions were all severe in homoiotransplantation; the thyroids were either completely destroyed or were on the way to destruction, both connective tissue increase and lymphocytic infiltration being very pronounced. In the syngenesiotransplantation on the other hand, the highest degree of destruction of thyroid and of increase in connective tissue was not usually reached even in the very unfavorable cases. In thirteen cases of this series transplantations were made of the organs of one child to father as well as to mother or of the organs of two brothers

to father or to mother. In seven cases the results in such pairs were the same or similar and in six they were different.

We may cite some examples in order to bring out certain facts relative to the behavior of various organs and tissues after transplantation. (*a*) *18 days*. Organs of child to mother. Both thyroid transplants well preserved. Acini lying close together, forming a ring. Epithelium low to medium; solid, retracted colloid. In one transplant the thyroid has the shape of an ellipsoid with normal host connective tissue and blood pigment cells in the center, such as we find in autotransplants, while in the other piece the thyroid has a round or oval shape, owing to an increased amount of fibrous tissue in the center, with which are mixed blood pigment cells. In the second transplant there has probably been more hemorrhage, with resulting increase in connective tissue and blood pigment cells. Penetrating the connective tissue are strands of fibroblasts and in addition large blood vessels and lymph vessels, filled with lymphocytes, enter the interior of this central fibrous portion of the thyroid. At the inner margin of the ring of thyroid acini and around parathyroid there is a partly diffuse and partly localized collection of lymphocytes; the lymph vessels are well filled with lymphocytes. In other places lymphocytes are lacking. Only in certain places is there a dense and usually localized lymphocytic infiltration. Some lymphocytes penetrate between the acini. Other parts of the thyroid behave like autotransplants. A few, but on the whole not many, lymphocytes invade also the parathyroid. In both transplants the parathyroid cells are full of mitoses. The cartilage transplant is normal and well preserved, especially the thin parts of the piece. It is surrounded by areolar and fat tissue and in places there is some connective tissue increase. There is no distinct lymphocytic infiltration. Grade 5.

(*b*) *20 days*. Organs of child to father. Of the uterus transplant only remnants are visible, namely, some well formed cuboidal epithelium surrounding the lumen; also serous coat. Intense lymphocytic infiltration in fat and connective tissue; lymph vessels studded with lymphocytes. Ovary: germ epithelium forms a cyst; interstitial tissue and medullary epithelial ducts preserved. Lymphocytic infiltration near germ epithelium cyst; lymph vessels studded with lymphocytes; otherwise in ovary moderate amount of lymphocytic infiltration. Grade 2.75.

(*c*) *21 days.* Organs of child to mother. Good thyroid ring; solid, good colloid. Medium or low epithelium. In center some fibrous tissue and blood pigment with large vessels, also lymph vessels filled with lymphocytes; very moderate lymphocytic infiltration around vessels. Ovary very well preserved; large follicles and many mitoses in granulosa cells. Good theca interna with capillaries around it. Small follicles and primordial follicles with eggs. Some degeneration of granulosa. Corpora lutea and interstitial tissue well formed. Germ epithelium cyst. Good muscle tissue. Tubes preserved. Fat tissue with some lymphocytes. Grade 5.50.

(*d*) *22 days.* Thyroid resembles lymph gland. Mainly from inner margin of center, but also from outside, large masses of lymphocytes enter septa of thyroid through vessels. Only remnants of acini without colloid are left; the remaining acini are either single or occur in parcels. Lymphocytes penetrate not only between but also into acini and destroy them. A somewhat greater part of parathyroid is preserved; in areolar tissue around it, strands of lymphocytes, also around perichondrium, in places, large collection of lymphocytes. Connective tissue is here newly formed and blood pigment cells infiltrate the tissue. Grade 2.25.

(*e*) *25 days.* Organs of child to mother. Thyroid transplant almost like lymph gland. Dense lymphocytic masses crowd lymph vessels. Great parts of thyroid destroyed. A number of acini with colloid still left; other acini without colloid. Lymphocytes overwhelm and destroy acini and connective tissue grows around them. Some remnants of hyaline vessels. Cartilage well preserved. A certain amount of perichondrial cartilage formation at end. Around cartilage, areolar tissue in which there are here and there moderate but distinct lymphocytic infiltration and some connective tissue increase. Cartilage transplant much better preserved than thyroid. Grade 2.5.

(*f*) *28 days.* Organs of child to mother. Thyroid and parathyroid behave like autotransplants. Cartilage surrounded by areolar and fat tissue; no connective tissue or lymphocytic increase. Bone preserved and also bone marrow with capillaries, osteoblasts, osteoclasts, megakaryocytes. Grade 6.

(*g*) *33 days.* From child to mother. Thyroid transplant with structure of autotransplant. Acini well preserved, joined close together. Solid colloid. Little fibrous tissue in center, but here and in

various places in periphery, lymphocytic infiltration. In certain places lymphocytes penetrate also between acini and enter the parathyroid accompanying the vessels; quite marked lymphocytic infiltration but only small parts of thyroid destroyed. Good parathyroid. Cartilage well preserved. At one end around necrotic cartilage perichondrial regeneration. Much fibrous tissue, but still some areolar and fat tissue around cartilage. In areolar and fat tissue a certain amount of lymphocytic infiltration especially around vessels. A slight mantle of lymphocytes around perichondrium; on the whole little lymphocytic infiltration. Grade 4.5.

COMMENT. These cases illustrate some interesting conditions. In the first case (*a*) the amount of connective tissue developing in the center of the two transplanted lobes of the same thyroid varies considerably, owing in all probability to accidental hemorrhage into one lobe, which led to more organization. In this case also numerous mitoses were found in the transplanted parathyroid cells, both transplants acting in this respect alike. Cases (*b*) and (*c*) are of interest because they represent different intensities of reactions against the transplants. In both instances there is a correspondence between the behavior of the host toward the different pieces. In (*b*) the reactions are severe; the uterus transplant is largely destroyed, and correspondingly only the most resistant parts of the ovary are preserved, namely, germ epithelium, medullary ducts and interstitial tissue; there is relatively much lymphocytic infiltration, while in case (*c*) where the thyroid is very well preserved, all parts of the ovary are in an excellent condition. In cases (*d*) and (*e*) the thyroid transplants both show an intense lymphocytic reaction; in one case there is also some connective tissue new formation between the acini; the parathyroid shows likewise a lymphocytic reaction, which is however less pronounced than that against the thyroid. The cartilage transplants, in particular the tissues surrounding the cartilage, show the least intense reaction, but there is in both kinds of transplants an increase in connective tissue and in lymphocytic infiltration. Case (*f*) shows again the correspondence between the reactions in different organs. Thyroid, parathyroid and cartilage behave like autotransplants; correspondingly bone and bone marrow are preserved; instead of being replaced by fibrillar connective tissue, we recognize typical bone marrow constituents, in particular megakaryocytes. The last case shows in a transplant thirty-three days

old, an auto-structure, but secondarily, attracted by syngenesio-toxins which gradually develop, lymphocytes begin to infiltrate the interacinar tissue. The cartilage shows corresponding changes.

Series III. Transplantation from Parents to Children

In this series fifty-five experiments were carried out; in twenty-six experiments the pieces were taken out between the eighteenth and twenty-fifth day and in twenty-seven experiments between the twenty-sixth and fiftieth day following transplantation; in two cases the examination took place thirteen and sixteen days after transplantation.

(1) *18 to 25 days after transplantation.* Auto-reactions or beginning lymphocytic infiltration in eight cases. Syngenesio-reactions with moderate lymphocytic infiltration in five instances and with considerable lymphocytic infiltration in six. A condition approaching homoio-reactions was found in seven cases. In the case examined at sixteen days, there was beginning lymphocytic reaction and in the case examined after thirteen days a homoio-reaction was obtained. Grade 6 in 1 case; grades 5.75 and 5.50 in 7 cases; grades 5.25 and 5 in 1 case. Grades 4.75 to 4 (incl.) in 6 cases; grades 3.75 to 3 (incl.) in 7 cases; grades 2.75 to 2 (incl.) in 4 cases, and below grade 2 in 2 cases. Average grade 3.9.

(2) *26 to 50 days.* Auto-reactions in three cases. Syngenesio-reactions with slight lymphocytic infiltration in two cases, with moderate lymphocytic infiltration in three cases, with marked lymphocytic infiltration in six cases. Homoio-reaction or a condition approaching it in thirteen cases. Grade 6 in 2 cases; grades 5.75 and 5.50 in 2 cases; grades 5.25 and 5 in 2 cases; grades 4.75 to 4 (incl.) in 5 cases; grades 3.75 to 3 (incl.) in 5 cases; grades 2.75 to 2 (incl.) in 4 cases; grades below 2 in 7 cases. Average grade 3.2. Total average grade in this series 3.6.

In fourteen cases we compared, in the manner described in the other series, the results obtained in pairs or triplets (transplantation from one parent to two or three children or from both parents to one child). In five cases the same or similar results were obtained while in nine cases the results were different.

We may comment on the findings in a few of our experiments in this series. (*a*) In a case examined nineteen days after transplanta-

tion, no trace of the thyroid transplant is found on microscopic examination. Uterine epithelium and muscle tissue are preserved, surrounded by dense fibrous tissue. There is fairly marked lymphocytic infiltration, especially in the muscle tissue. The fibrous tissue underneath the epithelium protects the latter, to some extent, from the invasion of the lymphocytes, although some lymphocytes are able to migrate through this layer. It is of interest that in this specimen a certain parallelism exists in the behavior of thyroid and uterus transplants; against both the reaction on the part of the host is marked. Of interest furthermore is the protective influence exerted by the layer of fibrous tissue underneath the epithelium. Grade 2.

(*b*) The findings were quite different in another case in which the transplants were likewise examined after nineteen days. Here thyroid as well as parathyroid are well preserved, and very large. The thyroid shows auto-structure. Lymphocytic infiltration is very slight. Correspondingly, Fallopian tubes and uterine tissue are also very well preserved, epithelium as well as muscle tissue. In the ovary transplant, well preserved follicles of various kinds, atretic follicles, interstitial gland and a germ epithelium cyst are found. Only a few small collections of lymphocytes observed in the ovarian stroma. Here again correspondence between the behavior of various transplants in the same host is noted. Grade 5.5.

(*c*) In a case examined after twenty-one days, we had transplanted two lobes of thyroid from the father to the same child. It is of interest that here the reaction against both lobes is the same. In both transplants there is much fibrous and loose connective tissue with fat in the center. Well developed rings of acini are surrounded on the inside and outside by considerable masses of lymphocytes, which penetrate a little into the parenchyma of thyroid and parathyroid. Some acini are surrounded by fibrous tissue. Lymph vessels are crowded with lymphocytes. Grade 3.5.

(*d*) In a specimen examined after twenty-six days, thyroid, parathyroid and cartilage transplants show the character of typical autotransplants; of special notice here is the finding of mitoses in epithelial cells of the parathyroid transplant. We have made similar observations in other instances. Grade 6.

(*e*) In another case in which cartilage and uterus were transplanted from the same mother as in (*d*) to another child, the areolar and fat tissue around cartilage show much fibrous thickening and

lymphocytic infiltration. In the uterus transplant there is likewise mainly fibrous tissue with very large masses of lymphocytes. A great difference is observed in the behavior of the two children toward organs of the same mother; but the different organs transplanted into the same individual show a corresponding reaction. Grade 1.5.

(*f*) In three rats examined fifty days after transplantation, the thyroid transplants exhibit a typical auto-structure; especially the center of the transplant shows these characteristics very distinctly. But soon lymphocytes begin to fill the lymph vessels, entering the center of the transplants and from here penetrating into the thyroid ring and destroying parts of the acini; other portions of the thyroid ring, which vary in size in different pieces, are still preserved. Some invasion by lymphocytes takes place from the outside, but this is of much less significance. The parathyroid is also somewhat infiltrated with these cells. The cartilage transplants show some fibrous thickening and lymphocytic infiltration. These cases illustrate the late attack, by masses of lymphocytes, on originally well preserved transplants, the center of the transplants serving as a base from which the invasion chiefly takes place. Grades 4.25, 4.25, 4.

Series IV. Transplantation from Grandchildren to Grandparents

Fourteen experiments were made in this series. Several pieces from various organs were transplanted in each case. Examination took place twenty-five to twenty-nine days following transplantation. Auto-reaction was obtained in three cases; syngenesio-reaction with slight lymphocytic infiltration in five cases, with moderate or more marked lymphocytic infiltration in three cases; conditions corresponding to or approaching homoio-reaction in three cases. Grade 6 in 3 cases; grades 5.75 and 5.5 in 4 cases; grade 5 in 1 case; grades 4.75 to 4 in 3 cases; grades 2.75 and 2.5 in 2 cases; and grade 1 in 1 case. Average grade 4.66.

The average grade in this series is very similar to that obtained in Series II (transplantation from children to parents); here the average grade was 4.5. In transplanting pieces of tissue from the same grandchild to grandfather as well as to grandmother, the reactions may be either the same or different in the two grandparents. If we

transplant pieces from two grandchildren into both grandparents, the reactions against the pieces may be the same in the grandfather as in the grandmother or one grandparent may react more favorably to the transplants from one grandchild and the other grandparent more favorably to the transplants from the other grandchild. We find therefore here the same variations which we found in pair-transplantations in the preceding series.

(*a*) In one experiment in which the examination occurred twenty-five days after transplantation, the organization of the center of the thyroid transplant is not yet complete; probably hemorrhage has taken place and delayed organization. Fibroblasts and vessels grow into the center; but they also form fibrillar connective tissue around the acini and separate them. Marked lymphocytic infiltration is associated with the connective tissue activity. Lymphocytes migrate into many acini and overwhelm them gradually; however, there are still many areas of acini well preserved. In this case there is at this late period still a formation of fibrillar connective tissue around acini. Of interest also is the good preservation of cartilage, bone and bone marrow; in addition, transplanted muscle is found consisting of muscle spindles with chains of nuclei. In the areolar and fat tissue, around cartilage and bone and between muscle fibers there is marked lymphocytic infiltration and some formation of fibrous tissue. Again we find correspondence in the behavior of the different transplants. Grade 4.

(*b*) We may cite also a second case, examined after twenty-nine days, in which the thyroid transplant has the typical elliptic shape of an autotransplant with little fibrous tissue in the center. There is increased lymphocytic infiltration at inner edge of the ring of acini; besides the diffuse lymphocytic infiltration in the center, lymphocytes penetrate a little between acini and collect as a larger mass at one pole of the thyroid. Cartilage and bone are well preserved, the former being surrounded by much areolar and fat tissue. Near the bone there is a column of regenerating cartilage cells, becoming necrotic next to the bone. Connective tissue penetrates the bone. Transplanted muscle is attached to the cartilage. Muscle spindles with nuclear chains are noticeable. Of interest in this case is the preservation of the proliferating zone of cartilage near bone and the regeneration of the transplanted striated muscle tissue. Grade 4·75·

Series V. Transplantation from Grandparents to Grandchildren

Twenty-four experiments were made in this series. In two cases the pieces were examined sixteen days after transplantation and a homoio-reaction was obtained. The other cases were examined between the twenty-second and twenty-seventh day and showed an approximate auto-reaction in 4 cases; syngenesio-reaction with slight lymphocytic infiltration in 3 cases, moderate infiltration in 6 and marked infiltration in 3 cases; homoio-reaction in 6 instances. Grade 6 in 1 case; grades 5.75 and 5.50 in 3 cases; grades 5.25 and 5 in 4 cases; grades 4.75 to 4 in 4 cases; grades 3.75 to 3 in 2 cases; grades 2.75 to 2 in 5 cases; grades 1.75 to 1 in 3 cases, and, if we include the two 16 day specimens, in 5 cases. The average grade in this series is 3.5 if we include the 2 cases examined 16 days after operation and 3.7 if we exclude these 2 cases.

In a number of cases we transplanted pieces of tissue from both grandparents into the same child or into several children. The reactions against the pieces from the two grandparents may differ in the same grandchild; in other cases they are about the same. The reactions against the two pieces from the two grandparents may differ in different grandchildren. The character of the donors (grandparents) as well as that of the hosts (grandchildren) influences the result.

The reactions obtained in this series (transplantation from grandparents to grandchildren) are more marked than in the preceding series, in which pieces were transplanted from grandchildren to grandparents. In both cases the average grades obtained are similar to the grades in the corresponding tissues exchanged between parents and children. As in the other series, so we find here also the reactions absolutely less severe in the cartilage than in the thyroid transplant. Lymphocytes are especially observed where there is a connective tissue increase. This combination of connective tissue increase and lymphocytic infiltration is more noticeable in the fat tissue surrounding the cartilage. In other cases both reactions may be independent. This may be observed especially in the thyroid, where lymphocytes not rarely invade secondarily a transplant which shows auto-structure and in which there is therefore no marked increase in connective tissue. In regard to the preservation of various tissues it is of

interest that in one experiment in which the tissues were examined twenty-five days after transplantation, cartilage as well as thyroid is well preserved; in this case bone and striated muscle tissue are likewise in good condition. Grade 5.75.

Discussion

1. If we compare the grades in syngenesiotransplantation in the guinea-pig with those in the rat we find throughout higher grades in the latter animal. The following figures prove this difference very clearly.

Series	Guinea-pig grade	Rat grade
I, brother to brother	3.6	4.66
II, children to parents	2.88	4.5
III, parents to children	2.6	3.6
IV, grandchildren to grandparents	2.6	4.66
V, grandparents to grandchildren	3.0	3.5

If we consider in transplantations in the rat the grades obtained in the earlier period up to the twenty-fifth day we find: in brother to brother transplantations, grade 5.29; children to parents, grade 4.4; parents to children, grade 3.9. The reason for these differences in grades in the guinea-pig and rat series is probably the different degree of inbreeding in the guinea-pig and rat strains used in these experiments. The families in the rat series were obtained almost invariably from strains propagated by the same breeder and the individuals which were mated were usually related to each other, although care was taken that they should not be closely related. In the guinea-pig in many cases individuals obtained from different breeders were mated. We found indication in our previous experiments that the results are influenced by such factors. It will be necessary in further experiments to analyze syngenesiotransplantation in families in which the individuals to be mated are definitely not related to each other.

2. In the guinea-pig as well as in the rat the grades are highest in transplantations from brother to brother. This fact is quite distinct in the guinea-pig and it is also definite in the rat, if we compare the figures for the different periods at which examination occurred. The pieces in the brother to brother transplantations were examined relatively late while the pieces in Series II (children to parents) and

in Series IV and V (exchange between grandparents and grandchildren) were examined at a relatively early date following transplantation. On the average, the grade decreases somewhat at later periods, but this is not invariably the case; it may remain about the same.

In the guinea-pig the difference between grades in Series II, III, IV and V is slight; it is somewhat more marked in the rat. Here the grades in Series II and IV, in which pieces from younger were transplanted to older animals, are better than in the reverse transplantations. Whether this difference in the results is significant or whether it is due to coincidence, is not certain. It is possible that older tissues call forth stronger reactions than younger tissues. On the other hand, there is reason for assuming that if the hosts are animals used soon after birth the reaction will be decreased in intensity; however, animals of this age were not used in these experiments.

3. In both guinea-pig and rat the grades in syngenesiotransplantation are intermediate between those in auto- and homoiotransplantation. This fact is definite in the guinea-pig and it is also distinct in the rat although in the rat transplantations a complicating factor arises consisting in the increase in grades of homoiotransplants in the later period of transplantation. This increase is due partly to a gradual adaptation between host and transplant in the case of cartilage transplantations, but when thyroid is used it is probably due mainly to the fact that we have to deal in the later period with syngenesio- rather than with homoiotransplants. This point is being still further investigated at the present time. However, this intermediate character of the grades is of a statistical character; it represents an average. In individual cases we find all transitions from grades characteristic of autotransplants to those characteristic of typical syngenesio- and in the end of homoiotransplants. In the exchange of tissues between grandparents and grandchildren we find the same variety of grades which we observe in the exchange of tissue between parents and children. It is evident that the individuality differential does not depend upon a single genetic factor. We may represent the individuality differentials in the following manner, which is arbitrarily chosen as far as the actual number of factors involved is concerned. It appears, however, not improbable that the whole genetic composition of an animal constitutes the individuality differential.

Ovum I of Female (mother) A	Spermatozoan I of Male (father) B
1, 2, 3, 4, 5, 6, 7, 8.	a, b, c, d, e, f, g, h.

Individuality differential of tissues of child I: 1, 2, 3, 4, 5, 6, 7, 8; a, b, c, d, e, f, g, h.

Ovum II of same Female (mother) A	Spermatozoan II of same Male (father) B
1, 2, 9, 12, 14, 16, 7, 8.	a, l, m, n, g, h, r, s.

Individuality differential of tissues of child II: 1, 2, 9, 12, 14, 16, 7, 8; a, l, m, n, g, h, r, s.

There are thus in the individuality differentials of child I and child II a number of identical factors and a number of factors in one child not represented in the individuality differential of the other. In different cases the number of identical and different factors will vary and accordingly the result of transplantation will differ. The number of strange factors in the individuality differentials of host and transplant may vary between 0 and 16. We may represent in a similar manner the individuality differentials of the tissues of one parent and child.

Mother: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.
Child: 1, 3, 5, 7, 9, 11, 13, 15; a, c, e, g, i, l, n, p.

If father and mother had each an entirely different genetic composition, there would be in every transplantation eight strange factors active, whether the transplantation was from parent to child or from child to parent. This would probably lead to a homoio-reaction. Syngenesio-reaction could only be obtained if father and mother had a certain number of factors in common. Under the same conditions the chances for a closer similarity between the individuality differential of host and transplant would be greater in the case of brother to brother than of parent to child or of child to parent transplantation. There should be no essential difference in the reaction observed in the case of parent to child transplantation and child to parent transplantation because in both cases the number of strange factors would be similar.

In this way it may be possible to account in a purely tentative manner for some of the facts in transplantation of tissues. However, we must keep in mind the fact that in addition to genetic factors which are of a specific character, there are other factors which can modify the growth of the tissues and which are of a more general nature. Thus age and perhaps pregnancy also may influence the result of transplantation in other ways than by their effect on genetic factors. Such secondary factors may to some extent alter the average grade of the various kinds of transplants.

4. If we compare the behavior of different organs in syngenesiotransplantations we find a correspondence in accordance with the identity of the relation between individuality differentials of host and transplant in the same experiment. But here again other factors of a secondary character come into play. While the individuality differentials in transplanted thyroid and cartilage are identical in cases of transplantations of these tissues from one animal to the same host the intensity of the lymphocytic reactions called forth by these two tissues differs considerably. This is apparently due to the less active metabolism of the cartilage and surrounding fat tissue and to the less active discharge of the chemical substances which characterize the individuality differential. In regard to the other differences in the reaction against cartilage and thyroid, we refer to our previous papers. The same correspondence is found in the case of bone marrow, uterus and ovary. On the whole, ovary is invaded to a less extent by lymphocytes than thyroid, but the lymphocytic reaction provides also in the case of the ovary an approximate quantitative measure of the relation between individuality differentials of host and transplant. The greater the divergence of differentials, in particular the greater the number of strange factors carried into the host with the transplant, the more severe the reaction becomes. If a certain intensity in toxic action is reached, then the more sensitive constituents of the ovary suffer first; they are destroyed. Thus no well developed corpora lutea may be found, and even ova and follicles suffer. The peritoneal endothelium which tends to form a cyst, the interstitial tissue and the medullary endothelial ducts are longest preserved. Bone marrow is preserved only in auto- and good syngenesiotransplantation; otherwise it is destroyed and replaced by fibrillar or myxoid connective tissue. Of interest is also the finding of well developed striated muscle tissue, when there is a similarity in individuality differentials, as late as approximately a month or longer after transplantation; at this time striated muscle is not found in cases of homoio-reaction. If favorable syngenesio-reactions are obtained in general in these transplants we may observe active mitotic proliferation in the parathyroid cells, while this is lacking under unfavorable conditions. Thus all organs are affected in a similar manner by the degree of relationship of individuality differentials of host and transplant. The lymphocytic reaction presents the finest quantitative determination of these

relations. If the toxic action becomes somewhat stronger than that found in favorable syngenesiotransplants, the behavior of the connective tissue is also affected and if a still more toxic effect is obtained, then the more sensitive tissues do not regenerate in a satisfactory manner following transplantation.

Having observed these reactions which allow an approximate quantitative analysis of individuality differentials, and the comparative gradations of behavior of various kinds of transplants under the influence of the contact substances given off by tissues (substances that develop a graded toxicity under controllable conditions), it would be of advantage to use for analysis in further investigations animals which have been inbred for generations and whose individuality differentials have thus been made more homogeneous.

Summary

1. An analysis of syngenesiotransplantation in the rat shows the intensity of the reaction to be intermediate between reactions in auto- and homoiotransplantations. This intermediate grade is a statistical average. In individual cases all degrees of variation may be found between conditions approaching, on the one hand, autoreactions and, on the other hand, homoio-reaction.

2. In both guinea-pig and rat the average of individuality differentials of donor and host shows the smallest discrepancy in brother to brother transplantation; it is greater in parent to children or children to parent transplantation. Exchange of tissues between grandparents and grandchildren gives results similar to exchange of tissues between parents and children.

3. There are some differences between the relative severity of reactions in transplantation from the older to the younger generation and in transplantation of the reverse kind; it is probable that these differences, which are not always in the same direction, are due either to coincidences or to secondary factors which, in addition to primary factors of a genetic character (individuality differential) determine the intensity of the reactions against the transplants.

4. In general the reactions against syngenesiotransplants in the rat are less severe than those in the corresponding syngenesiotransplants in the guinea-pig. This condition is probably due to a greater homogeneity in the genetic composition of the rats than of the

guinea-pigs used by us. A tentative genetic interpretation of our results in transplantation is given.

5. These investigations confirm our previous conclusions that in multiple simultaneous transplantations, the reactions against pieces of different organs derived from the same donor are relatively of the same strength because of the identity of the individuality differentials, although absolutely the reactions differ. The differences are due to secondary factors. On the basis of these secondary factors we may distinguish (*a*) organs like thyroid and parathyroid (in which variations in the intensity of lymphocytic and connective tissue reactions cause in the main the differences in the fate of the transplant); (*b*) organs like the ovary (in which, while the lymphocytic reaction occurs, it is usually not very pronounced, but in which under the influence of very severe homoio-toxins only the more resistant component parts of the organ survive); (*c*) less resistant tissues like bone marrow, striated muscle tissue and to some extent also unstriated muscle tissue; the latter tissue survives or regenerates completely after transplantation for a longer period only in the absence of the more severe homoio-toxins; and (*d*) very resistant tissues like cartilage and perichondrium.

EXPERIMENTAL GANGRENE PRODUCED BY DIETARY MEANS *

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In the course of a nutrition investigation [1] in which a large series of albino rats was used, an interesting pathologic condition of the tail of some of the animals was observed. Three groups were studied: a control group on a diet satisfactory for growth; a group stunted by feeding a food low in lysine [2] (Ration I, Table 1); and a third group stunted at the same level of body weight by underfeeding with a qualitatively adequate diet (Ration II, Table 1). Of the twenty-one animals on the last or limited calorie dietary régime, twelve developed lesions at the extremities of their tails, which, although varying in the length involved, bore close resemblance to each other and were beyond question of the same nature and due to similar causes.

This tissue abnormality was first noted in rats which were 82 days old † and which had then been on the diet in question for a period of thirty-eight days; it seemed to be a type of necrosis. It is unlikely that infection played a rôle in this lesion since the rats whose tails became involved were not segregated from the other rats used in the experiment. Of the twenty-one rats on a low calorie intake, more than half developed the lesion while not one of the remaining forty-four rats showed any tail necrosis, although the latter were in as close contact with the first group as the individual rats of the first group were with each other. Stunting alone does not explain the phenomenon since the rats which were kept at constant weight by being fed the food deficient in lysine did not develop any demonstrable changes in their tails.

In order to test the possibility of reproducing the above phenomenon as well as to obtain fresh material for histologic study, the experiment was repeated using fifty male rats which were 29 days old. Half of the animals were given Ration III (Table 1), similar to

* Received for publication September 2, 1926.

† At this age, a normal rat of the sex here used is almost half grown, having passed about one-eleventh of its total life span.

Ration II except that a different preparation of casein was used. Since underfeeding with this diet adds the possibility of protein starvation to energy insufficiency as a possible etiologic factor in the production of the tissue injury, the rest of the rats were given Ration IV, so calculated that while the total energy value of the food given was insufficient for growth, the actual protein intake was the same as that voluntarily eaten by a growing rat of the same weight when Ration III was fed. For example, a rat of 50 gm. body weight growing at the normal rate eats daily about 5 gm. of Ration II and thus ingests 0.9 gm. protein. When a rat is stunted by feeding only 3 gm. of Ration II, it receives only 0.5 gm. protein per day, an amount which, under the conditions of the experiment, may result in protein hunger. By increasing the content of protein to 30 per cent as in Ration IV, a 50 gm. rat given 3 gm. daily receives too small an energy allowance to grow but does obtain 0.9 gm. protein which would be sufficient for normal growth if all other dietary requirements were satisfied. By using restricted quantities of Ration IV, therefore, we are dealing with an uncomplicated energy deficiency.

TABLE 1

	Ration I *per cent*	Ration II *per cent*	Ration III *per cent*	Ration IV *per cent*
Gliadin	18	..	..	..
Casein	..	18	17†	30†
Starch	51	51	52	39
Lard	22	22	22	22
Cod liver oil	5	5	5	5
*Salt mixture	4*	4*	4*	4*

* Salt mixture of T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, 1919, xxxvii, 557.
† Casein washed with isoelectric water, dried and reground. Analysis showed 13.4 per cent nitrogen, 1.38 per cent ash, 3.04 per cent moisture.

The rats were kept in individual cages with free access to water. Vitamin B was supplied in the form of yeast powder of which each animal received 0.15 gm. every second day. The rats were weighed every other day and a sufficient amount of food given to each to maintain a body weight of approximately 50 gm. Of the fifty rats, three died in the course of the experiment.

Necrosis was first observed in the extremity of the tail of a rat 49 days old which had received the protein-rich diet (Ration IV) for a period of nineteen days. Subsequently other rats became affected. The lesions manifested themselves in much the same manner as

those of the preliminary experiment. The first definite evidence of necrosis in any rat fed Ration III appeared forty-three days after the beginning of the experiment when the rat was 72 days old. The phenomenon, then, is not of accidental occurrence but may be produced at will.

The initial sign of the lesion was the loss of hair from the tip of the tail followed by swelling and reddening at the tip — apparently a typical inflammatory reaction. Next a darkening of the discoloration appeared and then a blackening of the extreme end. Within three to five days, varying in different rats, the black "dot" increased in size and soon a considerable area appeared shrunken, wrinkled and of dark brownish black color. In at least one case the necrosis involved as much as 13 mm. of tail length. The affected region became progressively more desiccated and the outer surface of the tissue sloughed off in flakes of dry, hard scaly material. At the line of demarcation between the dried, darkened area and the normal tissue, there was a swollen and extremely red zone bounded by a ring of retracted skin beyond which was the necrosis proper. After twelve to fourteen days, the entire blackened material had sloughed away leaving for the tail end a swollen, red, blunt tip which was abnormally thick because of the swelling and also because this new tail end was a portion which had previously been an intermediate segment. The tip was now a disc-shaped surface with a hard, white, fibrous tissue core in the center surrounded by a reddened area.

In the preliminary experiments after a period of fifty days upon a low calorie diet, most of the rats were given access to the same food *ad libitum* and growth at an unusually rapid rate ensued. Those tails which had already developed blackened, dry, necrotic tips were not greatly altered by this change of diet. That area of the tail already involved became progressively more and more dried and the process of exfoliation of the outer layers of the necrotic tissue continued until the entire blackened mass had sloughed away. The redness of the new tail ends gradually decreased and no new areas developed. When an extremely small area of the tail was involved and the process consisted merely of a red, indurated tip, neither a progression nor a regression of the process followed.

After the main experiment had progressed for sixty-eight days, four arbitrary classifications were established in order to facilitate

the study of the process. In Group I were classified all rats with tails of the following descriptions: no visible change; normal appearance with a small white tip at the extremity; slightly coarser texture than normal with slight scaliness of the tip; depilation of tip; slight reddening or swelling of tip. These tails were normal or slightly abnormal.

Group II consisted of those rats whose tails manifested marked scaliness of end, irregularity in outline of end, swelling of the tip with bulbous end, reddening of swollen end, slight constriction near end (usually 2 mm. from end) with bulbous portion beyond and beginning of spotting of tail end — red or pink. These tails were somewhat more advanced.

Group III showed a black dot at end of bulbous portion, deep red or cyanotic spots at end and marked constriction with definite swollen bulb beyond. These tails were definitely abnormal.

Group IV included rats showing evidence of dry gangrene varying from one to three or more millimeters of dry, black, scaly tissue and extremely red bulbous ends with black extremities where varying amounts of necrotic tissue had already sloughed away. These tails showed severe necrosis.

Group I included twenty-two rats of which seventeen had received the diet containing the smaller per cent of protein (Ration III) and five rats which had received the diet containing the larger per cent of protein (Ration IV); Group II included eight rats in all, four on Ration III and four on Ration IV; Group III included six rats, one receiving Ration III and five, Ration IV; Group IV included eight rats, one receiving Ration III and seven, Ration IV. Since the groups showing the more advanced stages of necrosis were made up largely of animals provided with the protein-rich food, it appears that there is a correlation between the amount of protein in the diet and the extent of the necrotic lesion.

The same diets were continued for a further period of sixteen days. During this time the lesions progressed similarly to the advance previously noted and at the end of this period examination resulted in classifying four rats previously listed as Group I as now characteristic of Group II and two rats previously of Group II now as typical of Group III.

The process simulated in every respect a form of dry gangrene. The gradual desiccation and desquamation of the tissue indicated a

process of gradually lessened nutrition and finally a total absence of blood supply. It seemed justifiable to assume, therefore, that the described form of gangrenous necrosis of the caudal extremity can be produced by dietary means. It furthermore appeared that the increase in protein percentage of the food hastened the process rather than retarded it.

At the time of classification of the tail lesions into four groups, sixteen tails were amputated and microscopic sections were made using four rats of each group. In four of the cases where the tails were cut, blood counts were made. The number of red blood cells per cubic millimeter of blood varied between 8,356,000 and 9,696,000 — figures within the normal range for the rat.[3]

Microscopic Examination *

A piece of tail about 4 cm. in length was taken which included the necrotic tip and some of the adjacent normal tissue. It was immediately placed in a 10 per cent formalin solution. The block obtained was cut into four portions and cross-sections from each of the four pieces were made in order to give a more nearly continuous picture of the condition of the tissue, advancing from the apparently normal portions to those markedly affected.

Sections of Group I show the normal structure of the tail in those portions most distant from the site of the lesion. The center consists of the cartilage — a rim of cartilaginous tissue arranged in a circular fashion with the enclosed space composed of a lacework of fine tissue. Just dorsal to the cartilage is the artery and just below the artery, a vein. There are also three more veins, one below the cartilage and one on each lateral aspect. Along with the veins can be distinguished nerve bundles. Four muscle bundles extend longitudinally and are placed symmetrically about the cartilage of the center. Advancing outward toward the site of the lesion, there is one place where the intima of the artery has been lifted up and a homogeneous, pink-staining substance is present between the media and the lifted intima. The lumen of the artery can be traced through to the end of the tail. The veins are also patent. The most distal portion shows numerous capillaries. The cartilage is very well preserved throughout.

* The authors are indebted to Professor R. G. Hussey of the Department of Pathology for his aid in the study of the microscopic sections.

In the sections of Group II a few slides show hemorrhages in the walls of the arteries. The veins are engorged and the capillaries are dilated. The cartilage is less well preserved, some sections showing hollowed out portions where the central tissue has fallen out in the process of preparation of the slides. There also seems to be some increase in the thickness of rim of cartilage.

In Group III the arterial wall seems to be somewhat thickened and the outline of the vessels somewhat irregular. Some arteries are filled with a reddish, homogeneously stained substance. The veins in some sections are collapsed and appear to have somewhat thickened walls. No capillaries can be made out. The cartilage is poorly preserved with a thick rim and the inner network of loose tissue sloughed away.

Sections of Group IV contain arteries with quite thick walls. Some veins are collapsed and have thick walls while others are dilated and filled with a homogeneous pink-staining mass. At the tips of the tails there are many hemorrhagic capillaries. The cartilage is poorly preserved and consists of a thick rim surrounded by fibrous tissue with a hollowed out center.

In all sections, the muscle tissue near the extremity of the tail assumes a pink, homogeneous stain suggesting hyalinization. The nuclei of the muscle tissue are pyknotic. The terminal portions of the tails show the dilatation and engorgement as described.

Discussion and Summary

The origin of the gangrene here observed offers a field for speculation. In general, the causes of gangrene are tissue injury either chemical or mechanical, heat or cold, failure of the general health, circulatory obstruction, nerve disorder, the nerves involved being the vasomotor or possibly the trophic, or microbic infection.[4] Thus a variety of processes may bring about a gangrenous necrosis, the underlying factor in all being a diminution in the blood supply to the parts affected. However, the production of gangrene is often aided by more general disturbances of circulation or by decreased vitality of the tissues from other causes, as for example in the case of diabetes where gangrene of the feet is so common.[5] The tendency of gangrene to manifest an upward spread is also well recognized.[6] In the animals in which the described lesions occurred, a marked degree

of cachexia was produced and it is highly probable that the gangrenous processes observed were due to a principle similar to that recognized as the frequent cause of venous thrombosis in such cachectic conditions as cancer, chronic nephritis, prolonged suppuration and infantile marasmus. In all these cases it is believed that an enfeebled circulation plays an important etiologic rôle, although other factors such as changes in the intima of vessels may well contribute.[7]

The observations indicate that a method has here been evolved in which a form of gangrenous necrosis can be experimentally produced, the chief causative factor in the mortification apparently being the production and maintenance of a cachectic condition of sufficient severity to produce stunting in the experimental animal. Increase in percentage of protein of the diet appeared to accelerate the process. Since the rats showed no evidence of abnormality other than the excessive activity and the rangy appearance due to skeletal overgrowth frequently observed in animals subjected to similar dietary restriction, it would seem likely that the lesions of the tails were local ones. It was also noteworthy that the necrosis was progressive, extending upward by a repetition of the entire process and that the same series of changes manifested themselves repeatedly. The process extended upward not only from the normal tail end or the stump left after the sloughing of a mummified portion of tail but also from the stump left after traumatic amputation of a portion of tail.

It is unlikely that the rats suffered an appreciable degree of anemia since blood counts obtained from four different animals were well within normal limits.

In those cases where the animals were subsequently given free access to the food, the advance of the process was checked although, of course, the portions of tail manifesting definite anatomic change could not be restored to normal.

The microscopic sections showed the most profound changes in the cartilage of the tail. The reticulated structure present in the center of the cartilage in normal portions was absent in those parts near the section of tail which showed necrosis. The rim of cartilage, thin in normal portions, was much thicker and more darkly stained in the other portions. In those parts markedly involved there was a rim of fibrous tissue surrounding the cartilage. The vessels did not show

extreme changes but suggested thickening of the walls and showed engorgement in some sections and were collapsed in others. The muscle tissue showed hyalinization and pyknotic nuclei.

REFERENCES

1. Anderson, J. E., and Smith, A. H. *J. Comp. Psychol.*, 1926, vi, 337.
2. Osborne, T. B., and Mendel, L. B. *J. Biol. Chem.*, 1914, xvii, 325.
3. Jencks, Z. *Am. J. Physiol.*, 1922, lix, 240.
   Falconer, E. H. *Am. J. Physiol.*, 1926, lxxvi, 145.
4. Da Costa, J. C. Modern Surgery. Philadelphia, 1922, Ed. 8, 175.
5. McCallum, W. G. Text Book of Pathology. Philadelphia, 1918, 38.
6. Boyd, Wm. Surgical Pathology. Philadelphia and London, 1925, 69.
7. Conner, L. A. Thrombosis. Nelson Loose Leaf Living Medicine, iv, 576.

PATHOLOGIC CHANGES IN THE NERVES OF THE STOMACH WALL IN CASES OF CHRONIC GASTRIC ULCER *

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Although the literature on chronic gastric ulcer is enormous, only a few reports have been made on changes in the nerve tissue in the wall of the stomach adjoining the ulcer. Perman [1] found the nerves surrounded by increased perineural connective tissue, but never any textural thickening of the endoneurium. He observed a few breaks in the continuity of nerves caused by the ulcer. Inflammation of the perineurium was frequent and obvious degenerative changes within the nerves themselves rare. Similar observations were made by Nikolaysen [2] in 1921, and in the same year Askanazy [3] drew attention to something new, namely, that alterations of the nerves adjoining gastric ulcer are far from being always destructive; on the contrary they seem to possess a marked tendency toward a proliferative, regenerative activity. However, none of this work is based on a systematic study of a consecutive series of sections. I have, therefore, subjected thirty-four specimens of chronic gastric ulcer to a careful histologic examination.

The technic employed was as follows: fixation in 10 per cent formalin, embedding in paraffin, serial sections 5 to 7 microns thick; staining methods: hematoxylin and eosin, Van Gieson-Hansen, Mallory's aniline blue method for collagen, and Masson's three-color stain (acid fuchsin-phosphomolybdic acid-jaune métanil).

In most cases I found the nerve tissue pathologically altered (see Table I). The first thing which attracts attention is the abundance of nerve elements as compared with those in sections from normal stomachs. Both the number of nerve branches present and their size are increased. According to my experience the individual nerve

* Received for publication August 28, 1926.

This paper is an abstract of a monograph on the same topic of which the investigations were made in The Institute for Pathological Anatomy, University of Copenhagen. The monograph was awarded the prize medal of the University. The complete monograph is to be published in the Acta Pathologica Scandinavica, 1926.

TABLE 1

Alterations of nerves in gastric ulcer

Preparation number	Site of ulcer	Hyperplasia of nervous elements	Nerves passing into ulcer	Nerves embedded in dense connective tissue	Peri-neuritis	Neuromas		
						Periarterial	Auerbach	Diffuse
1a	Lesser curvature; 4 cm. from cardia	−	−	A few	(+)	−	−	−
1b	" " 6 cm. " "	++	Several	Many	+++	−	−	−
1c	" " 8 cm. " "	+	Many	Several	+++	−	−	−
1d	" " 10 cm. " "	++	Several	A few	+++	−	−	−
2a	Pyloric orifice	+++	−	Numerous	+	Multiple; situated in retroserous tissue	Multiple	−
2b	Lesser curvature; 3 cm. from pylorus	+	A few	−	−	−	−	−
3	Lesser curvature; middle	++	−	−	(+)	−	One solitary	−
4	Lesser curvature; 4 cm. from cardia	+	Several	Many	+	−	−	−
5	Lesser curvature; middle	−	−	A few	−	−	−	−
6	Lesser curvature; middle	+	−	Several	−	−	−	−
7	Near pyloric orifice	?	−	−	+	−	−	−
8	Lesser curvature	+	−	A few	++	−	−	−
9	Lesser curvature; middle	+++	Many	Numerous	+++	One solitary	Multiple; containing nerve cells	−
10	Near pyloric orifice	−	−	−	(+)	−	−	−
11	Near pyloric orifice	+++	−	−	+	−	−	−
12	Lesser curvature; 7 cm. from cardia	+++	−	Numerous	−	Multiple; situated in retroserous tissue	One solitary	−

13	Lesser curvature; middle	++	Several	−	−	−	−	−
14	Lesser curvature; middle	+++	−	Several	++	Propagating into base of ulcer	−	−
15	Lesser curvature; 5 cm. from cardia	(Diffuse neuroma)	−	Numerous	+	−	−	Filling up submucous tissue and muscular coat
16	Lesser curvature; middle	−	−	−	−	−	−	−
17	Lesser curvature; middle	−	−	−	(+)	−	−	−
18a	Lesser curvature; middle	(+)	Numerous	Numerous	+	−	One solitary	−
18b	" " "	−	A few	Numerous	(+)	−	−	−
18c	" " "	−	Very few	Numerous	−	−	−	−
19	Lesser curvature; middle	+++	−	−	+++	−	−	−
20	Near pyloric orifice	+	Several	Numerous	+++	−	−	−
21	Pyloric orifice	−	A few	−	−	−	−	−
22	Lesser curvature; near pyloric orifice	+	Several	−	+++	−	−	−
23	Lesser curvature; 7 cm. from pylorus	+++	Numerous	Several	++	−	−	−
24	Near pyloric orifice	+++	−	Several	++	−	−	−
25	Lesser curvature; middle	+	A few	Several	+++	−	−	−
26	Lesser curvature; near pyloric orifice	+++	−	−	++	−	Multiple	−
27	Lesser curvature; middle	+	−	Many	−	−	−	−
28	Greater curvature	+++	Numerous	A few	(+)	−	One solitary	−

LEGEND: Moderate Reaction +
Considerable Reaction ++
Intense Reaction +++

fibrils are not thicker, that is, a genuine hypertrophy of them does not occur. On the other hand, they do show an extremely strong power of resistance against destructive influences and a marked tendency to regenerative activity. In many of the cases examined, moderately large nerve branches are seen to pass through the granulation tissue at the base of the ulcer to terminate directly in the defect caused by the ulcer. Such nerves, apparently perfectly sound, appear, as it were, to be cut by the ulcer.

The behavior of the nerve cells around the ulcers is remarkable. Previous authors have constantly maintained that nerve cells do not occur in the cicatricial tissue. This is not true. Portions of the myogastric plexus extend through the severed muscle coat into the cicatrix and in certain cases may be found directly beneath the granulation tissue layer. Naturally, such nerve cells are pathologically changed. They are small, elongated and arranged in chains and are not unlike the degenerative cells described by Beneke as occurring in certain ganglion neuromas. The cytoplasm is frequently stained deeply (Van Gieson-Hansen) and is sometimes vacuolated. The nuclei are pyknotic, eccentrically located and rich in chromatin. This description includes all the cells considered to be still functioning as judged by the presence of intact nerve fascicles in the neighboring parts. Still more striking changes occur, however, such as blurred outlines of cells, chromatolysis and caryolysis. In certain cases tissue is found containing proliferating nerve cells (preparations 1, 2, 9, 13, 19 and 25) often combined with coexisting inflammatory changes.

The next group of observations concerns the relation of the nerves to connective tissue. The findings may be divided into three groups — (1) the simple passive incarceration of the nerve in the cicatricial tissue;(2) the increase of the perineural connective tissue caused by inflammation; and (3) the growth of interlacing connective tissue fibers within the substance of the nerve. (1) was found in about 50 per cent of the cases examined; (2) is absent in only three cases (10, 16 and 17); and (3) was found but once. On the whole the increase of perineural connective tissue and inflammation are closely connected. This inflammation is toxic or infectious in origin; it may be acute or chronic, the latter being more frequent in this series. The acute forms present the usual picture of emigration and exudation. The chronic form may be congestive or more frequently infil-

trative or sclerotic; any combination may occur. The chief changes are infiltration with lymphocytes and proliferation of the connective tissue. Since the nerves are resistant to inflammation, the changes may be designated as perineuritis in most cases. Rarely the perineuritis is complicated by neuritis; the picture then is essentially an interstitial inflammation, edema and cellular infiltration of the interfascicular tissue. The cells of Schwann are swollen and the vessels dilated and filled with leucocytes; a true suppuration is rare, but does occur.

Due to pressure as a result of the inflammatory processes, the nerves may either atrophy or proliferate excessively (Pitres, Vaillard and Laignel-Lavastine [4]). Thus it seems that the perineuritic changes constitute a connecting link to some forms of hyperplasia and more marked alterations of the nerve. I have not mentioned the purely parenchymatous types of neuritis, because I could not see them as the methods of staining were suitable for only the more obvious degenerative changes.

The majority of the cells in the perineuritic exudation are lymphocytes; occasionally a few plasma cells are found. As the inflammatory process includes the proliferation of fibroblasts, these cells are often numerous and thus make it difficult to determine how far the nerve cell chains extend into the surrounding granulation tissue. The inflammatory changes of the gastric nerves are best seen in the myogastric plexus and in the branches coming from the muscle coats. The perineuritic infiltration as a rule is confined to the proximity of the nerve, not extending into the surrounding tissue. The muscle coats and cicatricial tissue are not infiltrated with lymphocytes until the more chronic stages have been reached. This fact seems to me to confirm Perman's opinion that the lesion may be a secondary, ascending lymphangitis along the perineural lymphatics.

Undoubtedly the inflammation extends along the nerve branches from the ulcer outward; but does this extension take place continuously or does it jump from one place to another, leaving uninvolved tissue between? Perman believes the latter to be true and refers to finding nerves with unchanged perineurium near the ulcer, and inflamed nerves as far from the lesion as the lesser omentum. However, the starting point of the perineuritis is undoubtedly the base of the ulcer. The inflammation may start from the cut nerves or per-

haps more frequently from the numerous spaces between the split fascicles of the perforated muscle coat, where the nerve tissue of the myogastric plexus is always found. When the muscle coat is preforated, a turning point in the history of the ulcer has been reached. The toxic or infectious agents force their way through these spaces in the perforated muscle layers into the nerve tissue and the inflammation continues still farther along the nerves, out through the inner and outer muscle coats to the serosa and on into the omentum. I have followed these changes by means of complete serial sections and am convinced that extension of the inflammatory process is continuous.

The relation of vessels to the ulcer is also of importance. It is a well known fact that small arteries may end freely in the ulcer; the stump of such a vessel is often found thrombosed and surrounded by necrotic tissue. This necrotic zone may be followed through the submucosa and muscularis and represents another portal of entry for inflammation. On the whole, the perineuritic changes are remarkably frequent. In seven of the preparations they are rather slight, and in two entirely lacking. In three of the preparations (4, 9 and 12), I observed a fairly uncommon change in the perineural sheath. The cross-sections of the nerves show a central core surrounded by layers of tissue. This core may consist of regular nerve fibers or may be reduced to a fascicle of Schwann cells. At the periphery there is a gradual transition to unquestionable connective tissue fibrils, concentrically interlaced in a typical manner. Do these layers arise from the perineurium or from the sheaths of Schwann? Staining by Masson's three-color method justifies the view that the structures in question represent transformations of peripheral neuroglia. How this transformation takes place is still uncertain. New investigations have been planned to throw light on this problem.

The last and by far the most interesting of the proliferative changes of the nerves of the stomach are the neuromas. In preparation 15, the following can be found. At a distance of 1 cm. from the organ, the nerve branches from the lesser omentum begin to increase in size and number. They surround the blood vessels, branch out in continuously finer ramifications and are embedded in a meshwork of connective tissue. In the stomach wall there are large masses of nerve tissue, especially in the longitudinal muscle coat which has been split by hundreds of nerves. They vary in size from very large

nerve fibers to innumerable minute fibers, and they emerge in the compact tela submucosa. The stomach wall is interlaced in this manner for a space about 3 × 6 cm.; in an area a little larger than 1 cm. square, I counted 150 medium-sized nerves besides innumerable smaller ones. On the whole, the branches showed no conspicuous anatomic changes. This lesion is called a diffuse neuroma.

In preparation 9 there can be seen along a medium-sized nerve a small neuroma embedded in fibrillar connective tissue. The neuroma macroscopically appears as a spot the size of the head of a pin. Its component parts are arranged in an irregular manner and they penetrate between the remnants of the longitudinal muscle coat. The neuroma contains numerous small polygonal nerve cells which are deeply stained and show definite processes. The nuclei are small, pyknotic, contain ample chromatin and are centrally located. The nerve cells are embedded in fairly dense tissue and are scattered irregularly through the neuroma. In another series from ulcers of the same type, similar observations have been made, as in preparations 2a, 3, 12, 18a, 26 and 28. We are confronted with the occurrence of multiple small neuromas arising in the central portion of the cut nerves of the myogastric plexus. In every case there is a small central cicatricial neuroma and since these contain a special type of nerve cell, I suggest the name Auerbach neuromas.

In case 2a, a third type of central cicatrix neuroma occurs. Near the lateral wall of the large ulcer is a conglomeration of arterioles the size of a hazelnut kernel. Surrounding a group of eight small arteries in this mass are nerves running in various directions and embedded in a coarse meshwork of fibrillar connective tissue. Many of the nerve branches are subject to obvious inflammatory changes. Similar conditions have been found in preparations 9, 12 and 14. I have entered them in the table under the heading periarterial neuromas. Thus two fairly well defined types of cicatrix neuromas exist, the periarterial neuroma and the Auerbach neuroma, while the diffuse neuroma occupies a unique position.

I am of the opinion that no genuine tumors have been reported in the cases recorded, neither ganglion neuromas (Beneke[5]), nor "sympathomes" arising from persisting embryonal neuroblasts (Masson[6]). On the whole, my observations confirm Askanazy's theory that by these proliferative changes we are confronted with a type, the cicatrix neuroma (Virchow) akin to the amputation neuroma. A

total defect has taken place in the mucous membrane which will not regenerate. The defect is filled up by granulation tissue which later becomes scar tissue. In time the inflammatory phenomena will disappear. Within this young connective tissue exist remnants of the original tissue including the nerve. The cut nerves regenerate by growing out into the fresh, juicy and flaccid tissue which in time becomes scar tissue or cicatrix containing neuromas of the amputation type.

Are all these alterations which I have described specific for chronic gastric ulcer? In order to answer this I have investigated a large amount of control material, namely, ulcerated cancers of the stomach, cicatrices following gastro-enterostomies, ulcers and cicatrices occurring anywhere in the intestinal tract, and finally typical amputation neuromas of the femoral nerve. In these lesions I have found alterations similar to those already described: perineuritis, nerves embedded in connective tissue and neuromas. No alterations of nerves found in chronic gastric ulcer can be designated as specific if thereby is meant that corresponding changes in their ulcers can be demonstrated. The changes in question are therefore to be regarded as of a secondary nature.

Discussion

Will these secondary nerve changes explain certain symptoms of gastric ulcer? Among the marked symptoms pain is the most predominating. In view of the fact that the nerve branches of the myogastric plexus show almost constant and frequently intense inflammatory changes in the perineural sheath — changes that will create pain, soreness and hyperesthesia in the peripheral nervous system — it seems a justifiable assumption that we are here confronted with at least one of the pain-creating causes. Furthermore, we find exposed nerve branches and nerve cells in the necrotic zone. It is beyond any doubt that the hydrochloric acid will affect them, but how? We do not know the pharmacologic relations intimately enough to be able at present to draw any conclusions with certainty. There is a possibility both of stimulation and of paralysis. Most probably the influence of the acid will be to cause contractions, possibly spasms, and these again may cause pain. The compression exerted on the nerves by the contracting connective tissue in which they are embedded may likewise prove productive of pain.

The neuromas, however, play a different rôle. It is a well known fact that central neuromas may be the origin of the neurotrophic ulcers on the extremities. Cutting of the periarterial sympathetic connection allows the ulcer to heal. Wertheimer's[7] "énervation de l'estomac," cutting of all the nerves that pass to the stomach, has a singularly favorable influence on chronic gastric ulcers. Frequently central cicatrix neuromas will be met with as a secondary nerve alteration in gastric ulcer. Presumably then these neuromas may be the cause of trophic disturbances, thus acting as an additional cause in producing the chronicity of the ulcer.

Summary

1. The frequent occurrence of nerves in the immediate vicinity of gastric ulcer strikingly demonstrates the great power of resistance inherent in nerve tissue.

2. Contrary to what has been shown by previous writers, nerve cells are frequently found in the scar tissue of the ulcer.

3. The inflammatory manifestations are most frequently perineuritis, arising from ascending lymphangitis.

4. The perineuritis extends continuously.

5. The proliferative alterations of the nerve tissue proper appear as central cicatrix neuromas; these may originate in the myogastric plexus and will then contain nerve cells, or they may develop from the periarterial nerves and are then devoid of nerve cells.

6. None of the nerve alterations are specific for chronic gastric ulcer; consequently they may be considered secondary.

7. Perineuritis and the involvement of the nerves in contracting scar tissue may cause pain, the prominent symptom of gastric ulcer. The neuromas may be a contributing cause to the chronicity of the ulcer.

References

1. Perman. *Arkiv. f. Kirurgi*, 1919, li, 355.
2. Nicolaysen. *Deutsch. Ztschr. f. Chir.*, 1921, clxvii, 145.
3. Askanazy, M. *Virchows Arch. f. path. Anat.*, 1921, ccxxxiv, 111.
4. Pitres, Vaillard and Laignel-Lavastine. See Gilbert and Carnot, Nouveau traité de médecine et de thérapeutique. Paris, xxxvi, 1925.
5. Beneke, R. *Beitr. z. path. Anat. u. z. allg. Path.*, 1901, xxx, 1.
6. Masson, P. *Lyon. chir.*, 1921, xviii, 281.
7. Wertheimer and Latarjet. Innervation et énervation de l'estomac. Lyon, 1922.

DESCRIPTION OF PLATES

PLATE 10

FIG. 1. Preparation 12. Section through nerves near ulcer, each surrounded by tunics of transformed peripheral neuroglia. Section stained by Masson's three-color method. About × 160.

PLATE 11

FIG 2. Preparation 15. Transverse section through lesser curvature. In upper left-hand corner, remains of mucous membrane; in lower right-hand corner, arteries entering muscular coat. Wall of stomach crammed with nerve bundles of every size. About × 40.

FIG. 3. Preparation 3. Section through neuroma derived from the myogastrie plexus and containing numerous nerve cells. The black stripes are muscle fibers. Stained by Masson's method. About × 120.

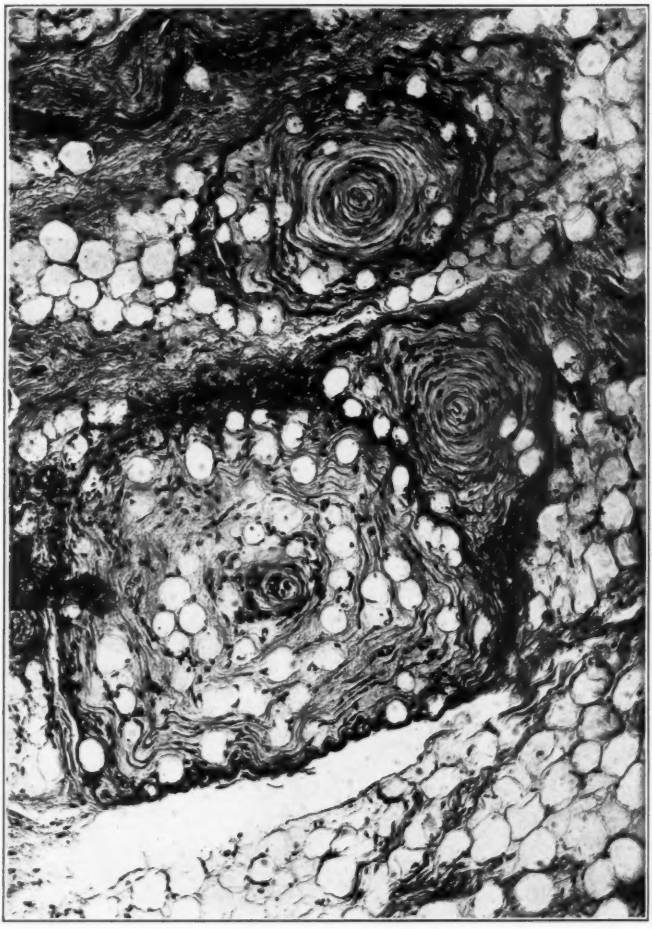
PLATE 12

FIG. 4. Preparation 2a. Periarterial neuroma near base of ulcer. The arterioles are subject to considerable pathologic changes. Stained by Masson's method. About × 80.

PLATE 13

FIG. 5. Preparation 9. Detail of neuroma derived from myogastric plexus, showing the extremely irregular course of the individual branches. Note the inflammatory phenomena. Stained by Van Gieson-Hansen's method. About × 300.

FIG. 6. Same preparation as in Fig. 4. Detail of neuroma within the cicatricial zone. The nerve fibers are interlacing the dense connective tissue. Stained by Masson's method. About × 120.



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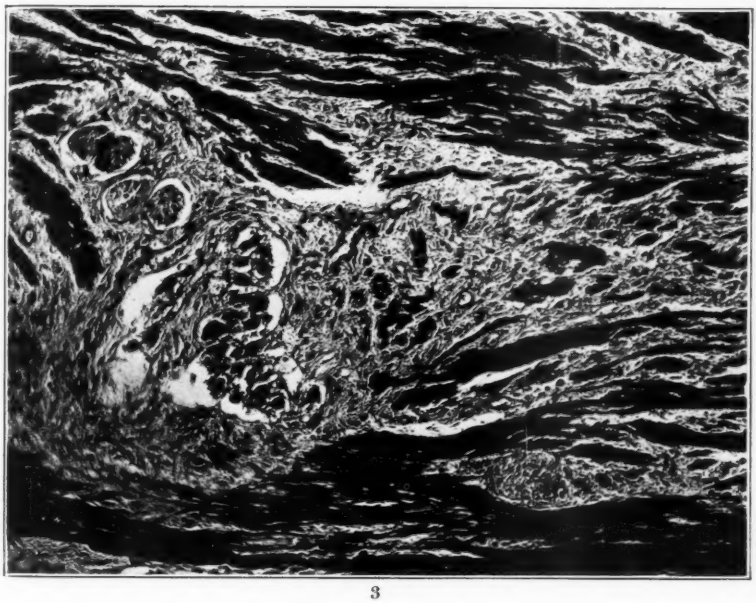

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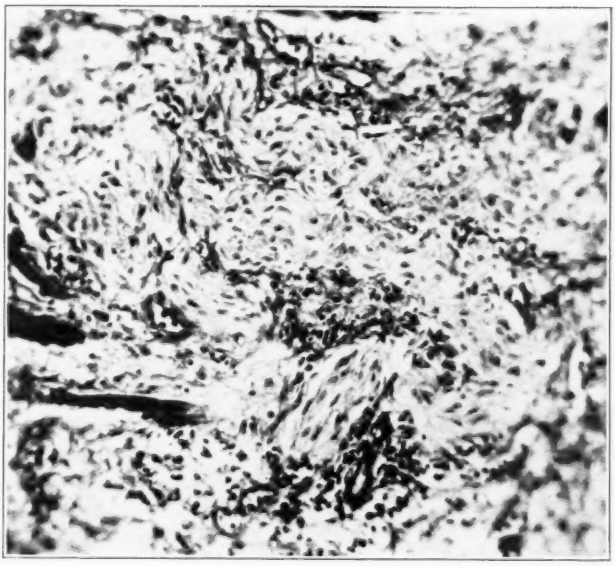

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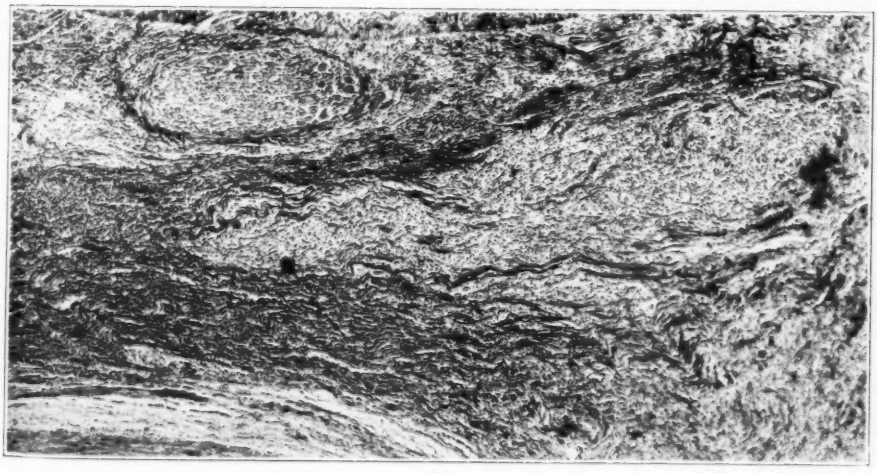

6

TUMOR AND FOREIGN BODY GIANT CELLS IN A FIBROSARCOMA OF THE UTERUS *

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AND

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The main purpose of this brief communication is to put on record, chiefly photomicrographically, a rapidly growing fibrosarcoma of the uterus containing two different types of giant cells in great numbers. This combination in a marked degree is rarely seen except in tumors arising in bone.

There are at least three types of giant cells which it is important to recognize and distinguish clearly from each other. The first results from multiple mitosis and occurs most often in rapidly growing tumors but occasionally under inflammatory conditions. The second is due to fusion of endothelial leucocytes to accomplish work which single cells are unable to perform. It is found in association with foreign bodies of all sorts such as lime salts, cholesterin and fatty acid crystals, cornified epithelial cells, and sutures and other foreign bodies introduced into the body. The third is formed by enlargement and direct division of nuclei and occurs in various cells under conditions of degeneration as in the epidermis, for example, following repeated freezing.

The tumor to be reported here illustrates the first two types of giant cells which are much more common and important than the third.

Gross Examination. The specimen consists of a symmetrically enlarged but somewhat nodular uterus measuring 15 × 8 × 10 cm. On section the lumen is filled with foul smelling, necrotic, greenish tags of tissue which are continuous with an apparently walled off, moderately solid, whitish tumor mass measuring 10 × 5 × 5 cm. Some of the necrotic material is calcified. In addition to the large mass there are multiple, small, dense, white nodules elsewhere in the uterine wall, evidently ordinary leiomyomas.

* Received for publication November 1, 1926.

The specimen was received within a few minutes after removal at operation and thin sections of tumor tissue were placed immediately in Zenker's fluid and other fixatives.

Microscopic Examination. The large tumor proves to be rapidly growing and at its periphery is infiltrating the muscle of the uterus. It is composed in places of spindle-shaped cells provided with very fine straight or slightly curving fibroglia fibrils. Between the cells run delicate wavy collagen fibrils in small numbers. These two types of fibrils demonstrate that the type cell of this tumor is the fibroblast. In other fields the cells vary from oval to spherical and few or no distinct fibrils can be found in connection with them. In still other areas the collagen fibrils are numerous and fused into thick hyaline strands which form a meshwork with the cells filling the spaces. These areas evidently represent the older parts of the tumor and have undergone retrograde changes.

Mitotic figures are present in great numbers in all areas but especially in those in which the tumor is growing most rapidly. Some of the mitoses are single but many are multiple and as a result giant cells containing large lobulated or multiple nuclei surrounded by delicate, lightly staining cytoplasm are numerous. Still more striking is the presence of many large foreign body giant cells often with one to two dozen or more nuclei embedded in rather dense cytoplasm. They occur not only singly but also in groups of various sizes. In places are small cavities, some lined with these giant cells and filled with serum, while others are filled with giant cells and look like small abscesses.

These foreign body giant cells are evidently at work dissolving the hyaline stroma left in places owing to necrosis of the tumor cells which formed it. In many areas the giant cells can be found closely applied to the intercellular substance. In this situation they have developed at the surface of contact a border of delicate short rods resembling cilia which stain intensely with eosin in eosin-methylene blue preparations of Zenker-fixed tissue and a deep blue with phosphotungstic acid hematoxylin. A similar layer of minute rods has been observed on the surface of osteoclasts where they are applied in the lacunae to bone which is undergoing solution. They may represent changed centrosomes as none can be found in these giant cells although they are present in the tumor cell type.

The foreign body giant cells seem to be formed entirely by fusion

of endothelial leucocytes which have been attracted into the tumor by the intercellular substance left by necrosing cells. All stages in their growth from small to large giant cells can be followed. Leucocytes adjoining a giant cell are first surrounded by cytoplasmic processes and then included within the cell. At first they stand out distinctly in the cytoplasm being surrounded by a light zone but later they fuse completely with it. Not infrequently an endothelial leucocyte in mitosis is taken into a giant cell in the same way and completes its cycle of development there before fusing with the cytoplasm.

The most striking feature of this case is the presence of the great number of foreign body giant cells in a tumor not involving bone in any way and due apparently to the attraction exerted on endothelial leucocytes by collagen which requires removal by solution in the same way that bone does. On this account, one point which has to be considered is the possibility that the attraction exerted for endothelial leucocytes in this tumor by the hyaline collagen is due to a deposit of lime salts in it. Calcification of the necrotic part of the tumor was noted on fresh examination but no evidence of lime salts could be found in sections taken from the living parts of the tumor and preserved in an alcohol-formalin mixture, but no silver test for calcium was made.

A second point of interest in this tumor is that it is easily possible to trace the formation and development of the foreign body giant cells by observing the way in which they not only fuse with one another but are incorporated into giant cells already formed. It is perfectly analogous to the formation of giant cells around agar injected subcutaneously as reported by Forbes [1] many years ago.

Recently, Cohen [2] and Lewis [3] have reported that they have been able to follow the formation of giant cells from endothelial leucocytes directly under the microscope in cultures of the blood.

Tumors of the uterus containing giant cells have been reported by several observers during the past few years and attempts have been made to draw prognostic conclusions from the presence of such giant cells without, however, precise statement of the nature of the cells, *i. e.*, whether they are true tumor or foreign body giant cells. The distinction is very important because multinucleated tumor cells indicate rapid growth and therefore marked malignancy while those of the foreign body type have an entirely opposite significance

so far as they themselves are concerned. All they indicate is an attempt to dissolve something which the endothelial leucocytes individually are incapable of accomplishing. They, therefore, signify an attempt at repair.

Summary

A fibrosarcoma of the uterus containing tumor and foreign body giant cells in great numbers is reported.

The tumor giant cells are formed from multiple mitoses and indicate rapid growth and malignancy.

The foreign body giant cells are evidently due to fusion of endothelial leucocytes attracted into the tumor by the intercellular substance left by necrosing tumor cells. The leucocytes are attempting to dissolve the hyaline collagen and have fused for this purpose. Along the surface where they are applied to it a layer of minute rods is formed, which are perhaps altered centrosomes. Osteoclasts present the same structure.

REFERENCES

1. Forbes, Alexander. The origin and development of foreign body giant cells. *J. Med. Res.*, 1909, xx, 45.
2. Cohen, Mortimer. Observation of formation of giant cells in turtle blood cultures. *Am. J. Path.*, 1926, ii, 431.
3. Lewis, W. H. Giant cells of the blood. *Science*, 1926, lxiv, Supplement x.

DESCRIPTION OF PLATES

Plate 14

Fig. 1. Spindle cell portion of tumor showing delicate fibrillar intercellular substance between the cells. Three mitoses present. × 500.

Fig. 2. An older part of the tumor; the intercellular fibrils are fused to form a hyaline meshwork. Mitoses numerous. × 500.

Plate 15

Fig. 3. Two mitoses, one large, the other of normal size; also tumor and foreign body giant cells. × 750.

Fig. 4. A multiple mitosis and tumor and foreign body giant cells. × 1000.

Plate 16

Fig. 5. A large group of foreign body giant cells. × 100.

Fig. 6. Several foreign body giant cells; one large tumor giant cell at right. × 500.

PLATE 17

FIG. 7. A large foreign body giant cell in center applied to collagen which it is dissolving. A crescent of short rods at line of junction. × 750.

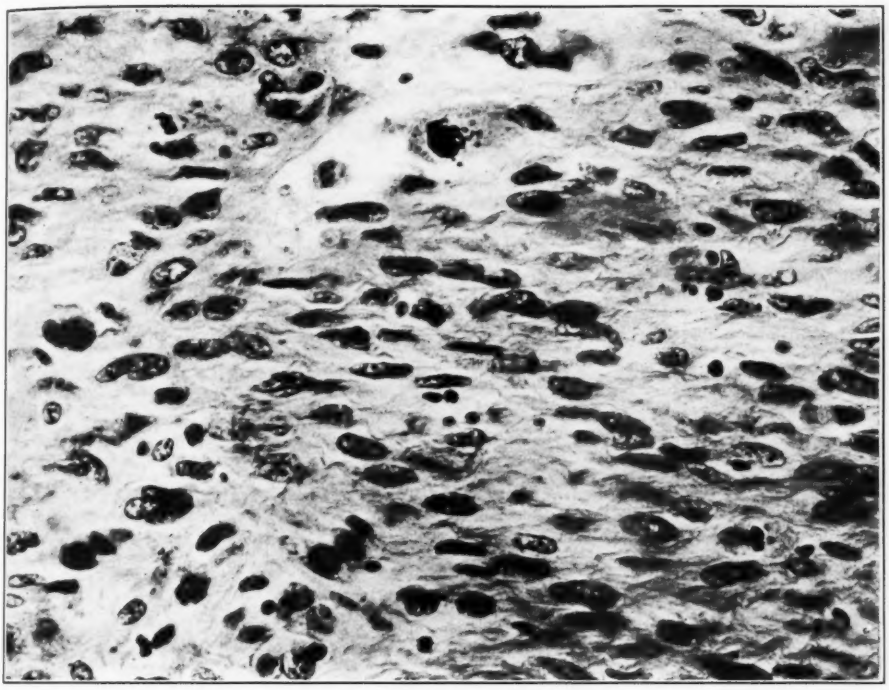
FIG. 8. A giant cell on right almost encircling a mass of collagen. Line of junction only moderately well defined by minute rods. On left, three foreign body giant cells, a large mitotic figure and a tumor giant cell. × 750.

PLATE 18

FIG. 9. A foreign body giant cell which has incorporated two endothelial leucocytes, one in mitosis. × 750.

FIG. 10. An endothelial leucocyte in mitosis in a foreign body giant cell. × 750.

FIG. 11. Endothelial leucocytes, of which one is in mitosis, just beginning to fuse around the remains of a small mass of agar injected subcutaneously. × 1000.



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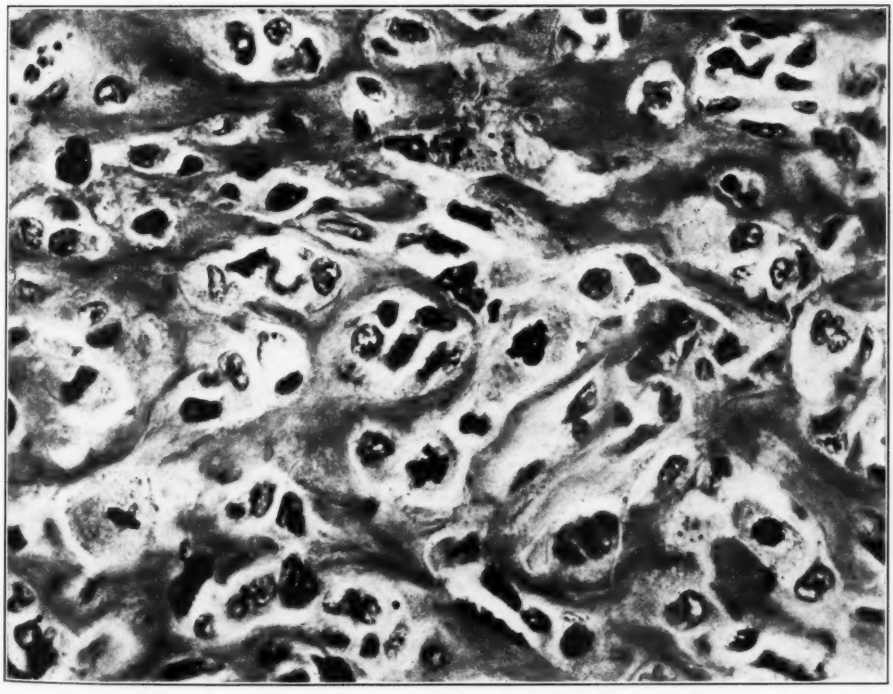

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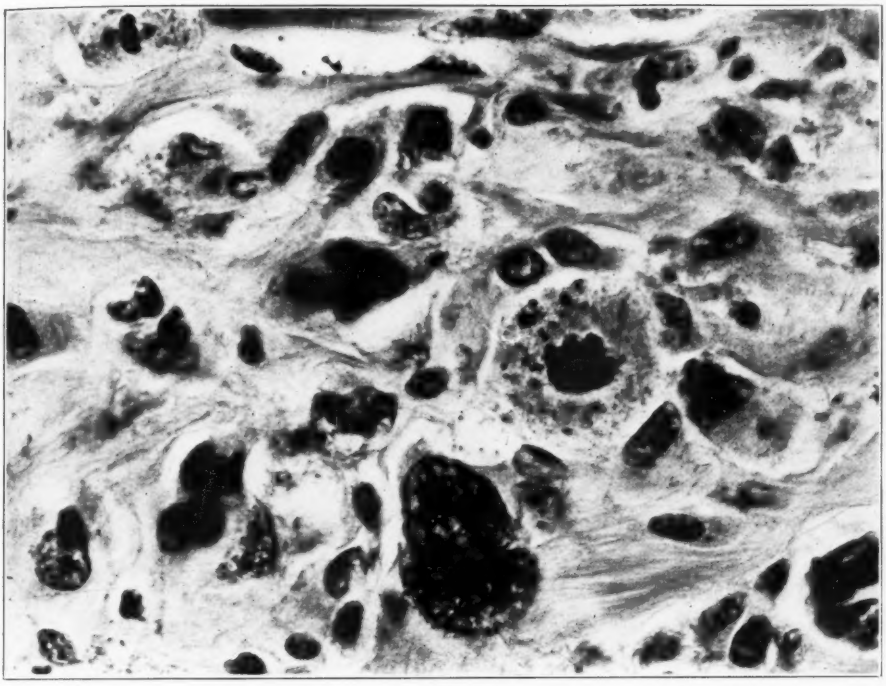
Mallory and Stewart Tumor and Foreign Body Giant Cells



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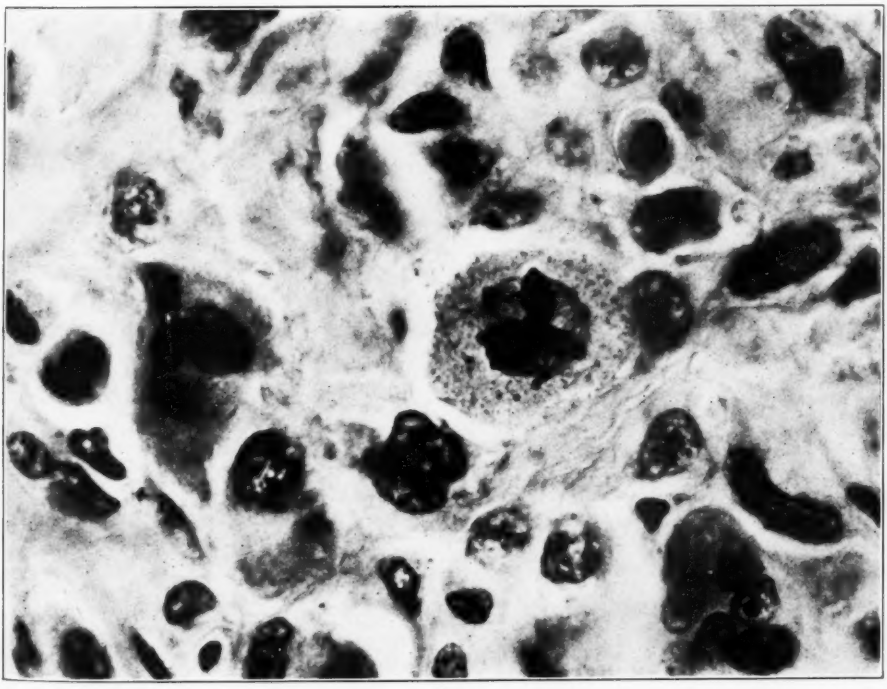

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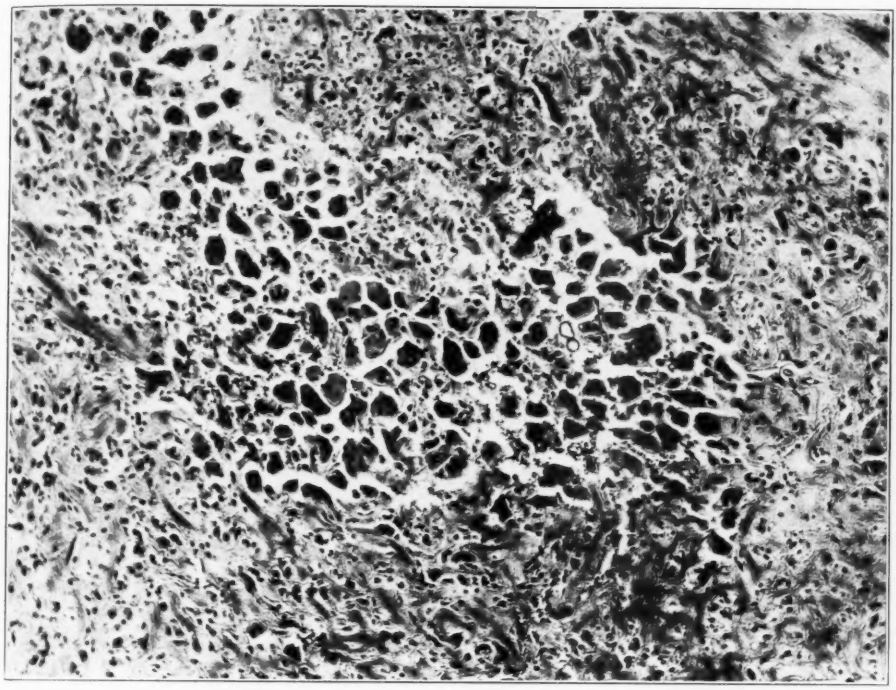

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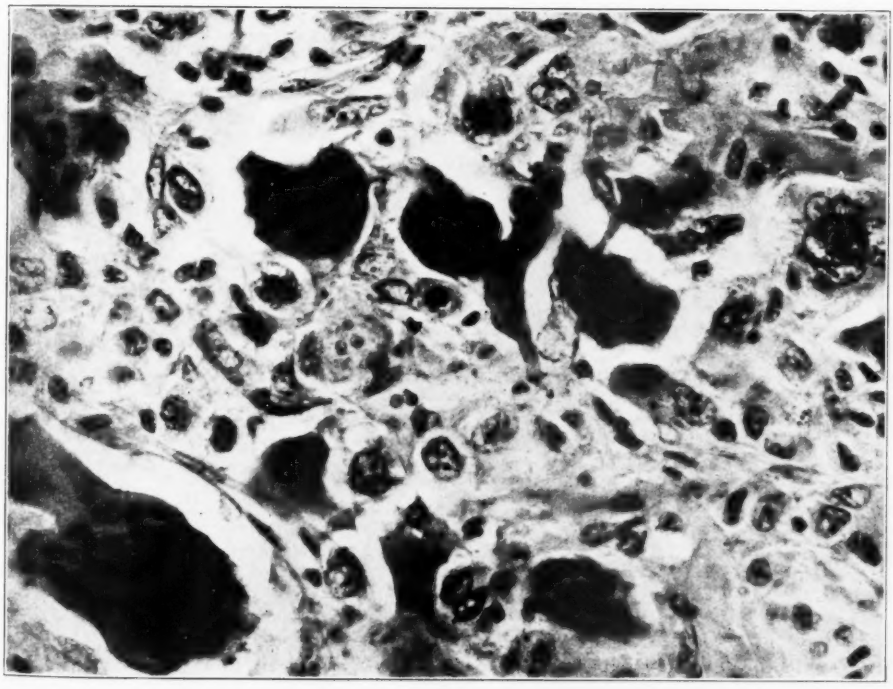

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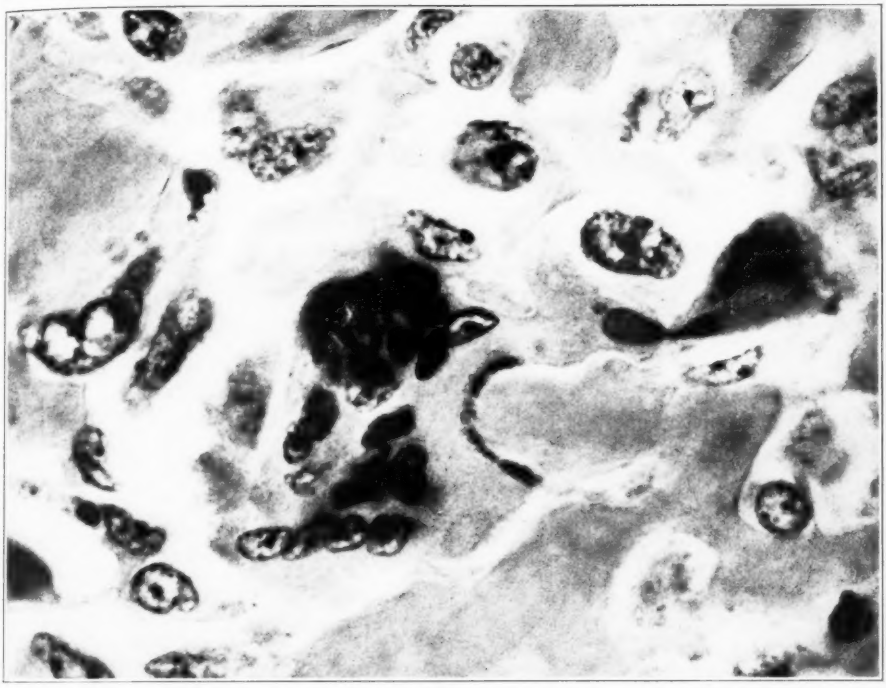
Mallory and Stewart Tumor and Foreign Body Giant Cells



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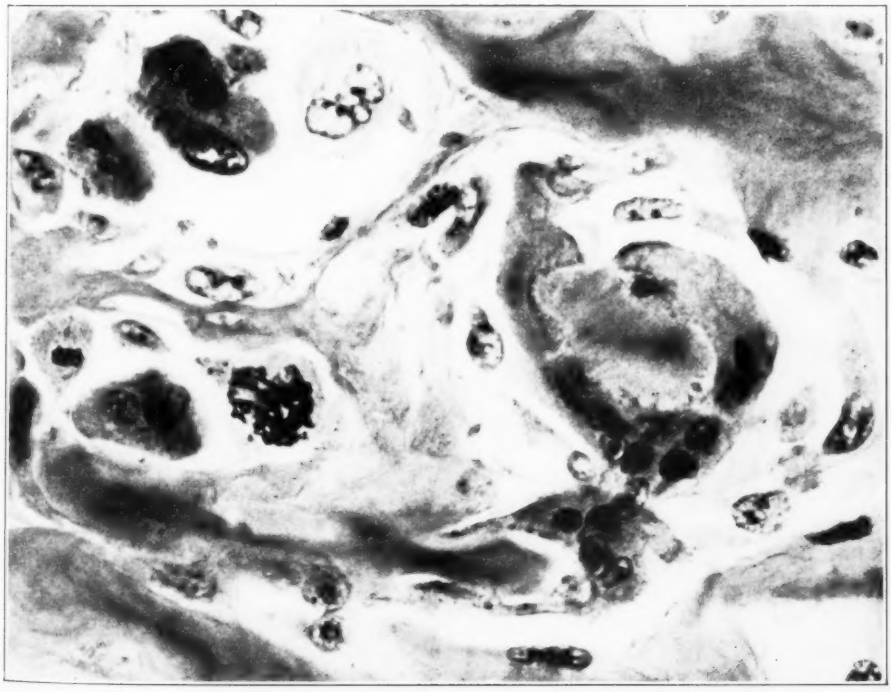

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Mallory and Stewart Tumor and Foreign Body Giant Cells

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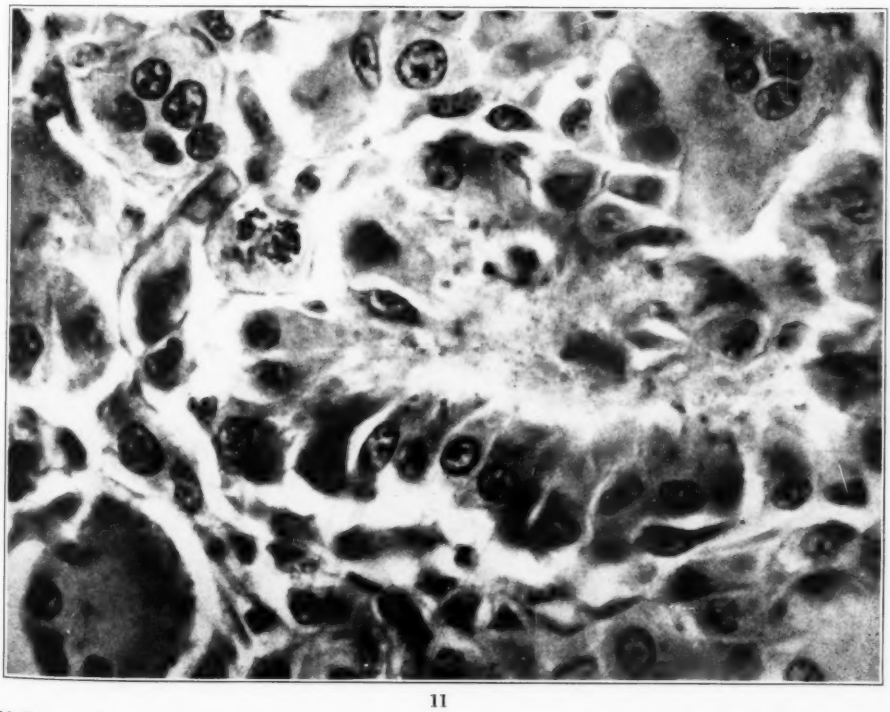

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Mallory and Stewart Tumor and Foreign Body Giant Cells

SPONTANEOUS INTRACAPILLARY GLOMERULONEPHRITIS IN THE RABBIT *

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At the twenty-fifth annual meeting of the American Association of Pathologists and Bacteriologists held in Washington, D. C., May 5 and 6, 1925, we reported [1] the presence of acute intracapillary glomerulonephritis as occurring in four out of eleven rabbits following the subcutaneous injection of 0.5 to 1 gm. of metallic zinc in fine powder form. The rabbits had been bred in the laboratory from good stock, there had been no epidemic of any sort so far as we were aware and no change in the character of the food. Moreover, the kidneys from several hundred rabbits had been examined in the course of other experimental work extending over a number of years and no similar lesion had ever been recognized. There is also a wide spread feeling among laboratory men that this type of lesion does not occur spontaneously. We, therefore, accepted our experiments as reliable and reported them accordingly.

For over a year since then we have carried on a series of experiments with zinc, employing not only several of its salts but repeating the original experiment of injecting the metal in fine powder form subcutaneously, both in larger doses than before and over a much longer period of time. The metal dissolves more or less readily in the tissues owing to the lytic action of the leucocytes attracted to it and causes a slight local reaction but produces no significant effect on the kidney. We, therefore, publish this acknowledgment of our error in hopes that it may save some one else from drawing a similar wrong conclusion.

The type of lesion which occurred deserves to be put on record although only the acute stage of it has been observed. The process starts with proliferation of the lining endothelium in the capillaries in one or more of the lobules of the glomerular tuft. Mitotic figures are fairly numerous. Sometimes two can be found in a section of a single glomerulus. The proliferation of the endothelium leads to occlusion of the capillaries and enlargement of the lobule. Some-

* Received for publication November 1, 1926.

times only one or two lobules are involved but as a rule the entire tuft is affected. The process is distributed uniformly throughout the whole kidney indicating that it is of toxic origin, not in foci as would be the case if it were due to the immediate presence of an infectious agent.

Counting the nuclei in sections of similar thickness and running through the middle of glomerular tufts showed two to three times as many in the affected glomeruli as in normal control ones. In addition to the glomerular lesion more or less degeneration of the renal epithelium was found, namely, hyaline droplet formation, necrosis and sometimes calcification. The tubules contained casts and occasionally showed regenerating epithelium.

We can offer no suggestion as to the cause of this glomerular lesion. During the course of our later experimental work, while studying kidneys under the oil immersion lens, we several times ran across mitoses in the capillary endothelium of the glomeruli. They may represent the early stage of this type of lesion but were evidently not due to the action of zinc because we found them occasionally in other rabbits also.

The type of lesion described here is entirely different from the one which is so often present in rabbits' kidneys and which leads to scarring (pitting) of the cortex. The irregular distribution of the latter would seem to indicate that it is of infectious origin.

Conclusion. Acute intracapillary glomerulonephritis sometimes occurs spontaneously in the rabbit.

REFERENCE

1. Mallory, F. B., and Parker, Frederic, Jr. Experimental intracapillary glomerulonephritis. *Am. J. Path.*, 1925, i, 541.

DESCRIPTION OF PLATE

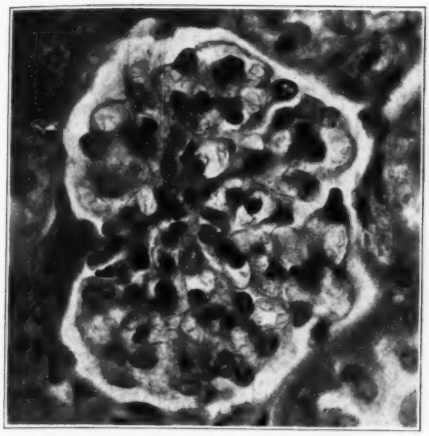
PLATE 19

FIG. 1. Normal glomerular tuft in kidney of rabbit. × 500.

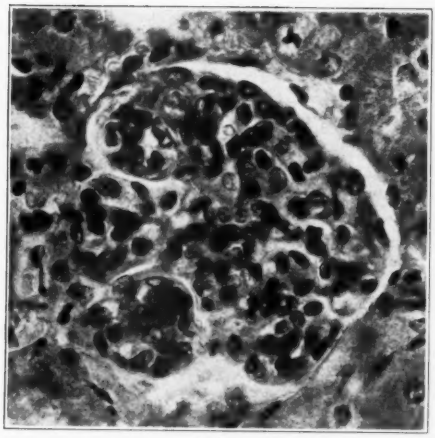
FIG. 2. Mitosis in endothelial cell lining capillary of tuft in early stage of lesion. × 1000.

FIG. 3. Diaster in tuft in well developed lesion. × 1000.

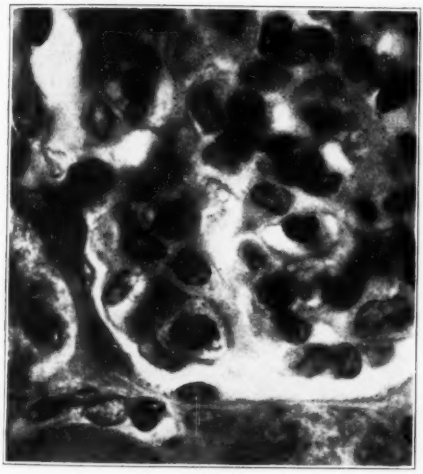
FIGS. 4, 5 and 6. Well marked lesions showing occlusion of the capillaries owing to proliferation of the endothelial cells, enlargement of some of the lobules and marked increase in the number of nuclei as compared with the normal tuft in Fig. 1. × 500.



1



4



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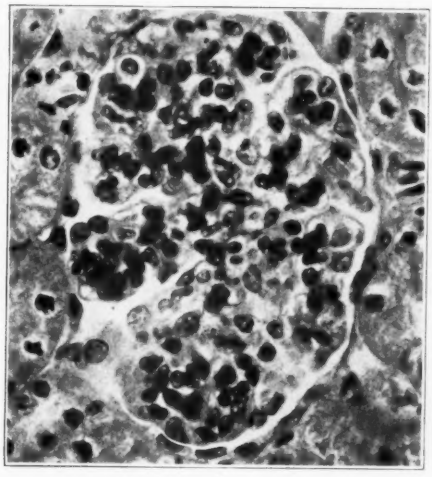

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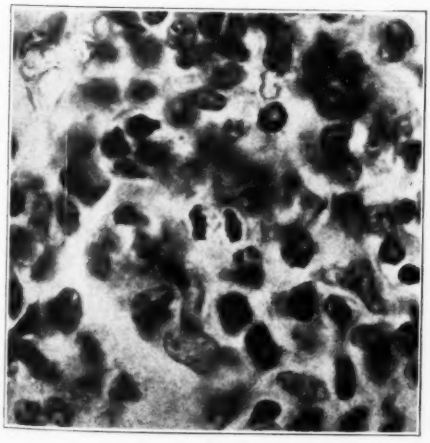
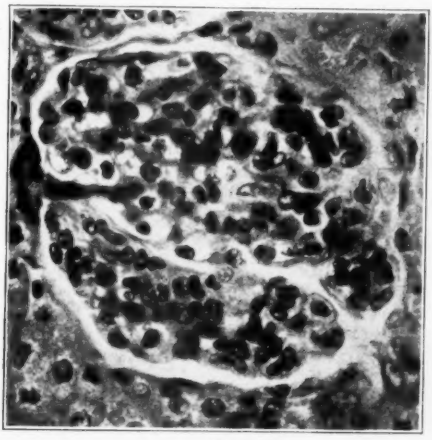


3



6

Mallory and Parker

Glomerulonephritis in the Rabbit